Fermentable carbohydrates and functional gastrointestinal symptoms in patients with inactive inflammatory bowel disease

Cox, Selina Rose

Awarding institution: King’s College London

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Fermentable carbohydrates and functional gastrointestinal symptoms in patients with inactive inflammatory bowel disease

Selina Cox

November 2018

A thesis submitted for the degree of

Doctor of Philosophy in Nutritional Sciences

Department of Nutritional Sciences

Faculty of Life Sciences and Medicine
To my family and Marc
Abstract

Many patients with inactive inflammatory bowel disease (IBD) continue to experience functional gastrointestinal symptoms (FGS) despite minimal objective evidence of GI inflammation, with a significant impact upon health-related quality of life. The low FODMAP diet is effective for the management of irritable bowel syndrome (IBS) and retrospective and prospective uncontrolled studies indicate that it may reduce FGS in patients with inactive IBD. However, this is yet to be confirmed in a randomised placebo-controlled trial. The low FODMAP diet reduces abundance of immune-modulatory Bifidobacteria and *Faecalibacterium prausnitzii* in IBS, alterations that may have clinical implications were they to occur in IBD.

The aim of this thesis was to establish the role of FODMAPs in FGS in IBD. Firstly, a case-control study was conducted to establish FODMAP intakes in patients with active IBD, inactive IBD with FGS and inactive IBD without FGS compared to healthy controls. This showed that patients with inactive IBD with FGS had lower GOS and polyol intakes than healthy controls, although whether FODMAPs induce symptoms in patients with inactive IBD remained unclear.

Therefore, a randomised, placebo-controlled re-challenge trial was designed to establish the effects of pure FODMAP challenges on GI symptoms. A pure fructan challenge induced FGS in patients with inactive IBD, proving FODMAPs as possible inducers of FGS in IBD. However, this trial did not establish efficacy of the low FODMAP diet compared to a placebo diet.

Therefore, a randomised controlled trial was conducted to investigate the effect of the low FODMAP diet on FGS in patients with inactive IBD. Patients with Crohn's disease and ulcerative colitis with minimal GI inflammation were randomised to either low FODMAP or placebo sham dietary advice, which was followed for 4 weeks. At end of trial, significantly more patients reported adequate relief of GI symptoms in the low FODMAP diet group (14/27, 52%) than the sham diet group (4/25, 16%; P=0.007). There was a greater reduction in IBS Severity Scoring System score in the low FODMAP diet group (-67 SD 78) compared to the sham diet group (-34 SD 50) although this failed to reach significance (P=0.075). There was significantly lower *Bifidobacterium longum* (P=0.006), *Bifidobacterium adolescentis* (P=0.005) and *Faecalibacterium prausnitzii* (0.038) following the low FODMAP diet compared to sham diet. Reassuringly, inflammatory markers and gut-homing T-cells were not different between the diet groups.

This thesis has established that patients with IBD restrict dietary fermentable carbohydrates, and that certain types induce FGS in patients with inactive IBD. Additionally, a low FODMAP diet improves FGS in these patients but reduces immune-regulatory GI bacteria abundance, a finding that requires further investigation.
Acknowledgements

My first thanks must go to my primary supervisor, Professor Kevin Whelan, for inspiring, encouraging and challenging me throughout the past 3 years and for helping me realise and achieve my potential. This would not have been the experience it has been without you and I have learnt so much from you. Thank you also for making me laugh even on the toughest days and for making meetings so enjoyable! Thank you to Dr Peter Irving and Dr Miranda Lomer for your invaluable input and to Professor James Lindsay for your shrewd insight and enthusiasm. Thank you to Dr Andrew Stagg for guiding me through the immunology aspects of this project.

Thank you to the Kenneth Rainin Foundation for supporting the work within this PhD, especially the metagenomic sequencing and immunology that answered several important research questions.

Thank you to Clio Myers for teaching me the practical aspects of recruiting to the re-challenge trial, and to everyone in the lab at Queen Mary, especially Dr Neil McCarthy and Inva Hoti, for teaching me flow cytometry and for patiently answering my many questions. I also extend a thank you to all those at INRA who contributed to the metagenomic sequencing analysis and kindly accommodated me for several days during my visit. I particularly owe a thank you to Sébastien Fromentin for tirelessly answering my bioinformatic queries. Thank you also to Robbie Gray for your assistance with short-chain fatty acid analysis and to Barry Hudspith for kindly overseeing the calprotectin ELISA. Thank you to Charlie Howard and Alice Wright for entering the diet diary data. I am also extremely grateful to Dearbhaile O’Hanlon for selflessly helping me identify suitable patients.

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Publications and award arising from this PhD

Publications


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**Conference abstracts**


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**Awards**

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2017 Nutrition Society Summer Summer Conference, Best Oral Communication: Fermentable carbohydrates (FODMAPs) as triggers of functional gastrointestinal symptoms in patients with inflammatory bowel disease: a randomised, double-blind, placebo-controlled, cross-over, re-challenge trial
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>ANCOVA</td>
<td>Analysis of covariance</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>AR</td>
<td>Adequate relief</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BSFS</td>
<td>Bristol Stool Form Scale</td>
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<tr>
<td>CARD15</td>
<td>Caspase recruitment domain-containing protein 15</td>
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<tr>
<td>CD</td>
<td>Crohn's disease</td>
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<tr>
<td>CDAI</td>
<td>Crohn's disease activity index</td>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CMC</td>
<td>Carboxymethylcellulose</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
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<td>DC</td>
<td>Dendritic cell</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<tr>
<td>DP</td>
<td>Degree of polymerisation</td>
</tr>
<tr>
<td>DRV</td>
<td>Dietary reference value</td>
</tr>
<tr>
<td>EEN</td>
<td>Exclusive enteral nutrition</td>
</tr>
<tr>
<td>ESR</td>
<td>Erythrocyte sedimentation rate</td>
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<tr>
<td>FCP</td>
<td>Faecal calprotectin</td>
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<tr>
<td>FGID</td>
<td>Functional gastrointestinal disorder</td>
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<tr>
<td>FGS</td>
<td>Functional gastrointestinal symptoms</td>
</tr>
<tr>
<td>FISH</td>
<td>Fluorescence in situ hybridisation</td>
</tr>
<tr>
<td>FMT</td>
<td>Faecal microbiota transplantation</td>
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<tr>
<td>FODMAP</td>
<td>Fermentable oligosaccharides, disaccharides, monosaccharides and polyols</td>
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<tr>
<td>FR-QOL</td>
<td>Food-related quality of life</td>
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<td>GI</td>
<td>Gastrointestinal</td>
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<td>GOS</td>
<td>Galacto-oligosaccharides</td>
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<td>GRS5</td>
<td>Gastrointestinal symptom rating scale</td>
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<td>GWAS</td>
<td>Genome-wide association studies</td>
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<td>HBI</td>
<td>Harvey-Bradshaw index</td>
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<td>HR-QOL</td>
<td>Health-related quality of life</td>
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<td>IBD</td>
<td>Inflammatory bowel disease</td>
</tr>
<tr>
<td>IBS</td>
<td>Irritable bowel syndrome</td>
</tr>
<tr>
<td>IBS-SSS</td>
<td>IBS Severity Scoring System</td>
</tr>
<tr>
<td>ILC</td>
<td>Innate lymphoid cell</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>MAdCAM-1</td>
<td>Mucosal addressin cell adhesion molecule-1</td>
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<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>M-SHIME</td>
<td>Mucosal simulator of the human intestinal microbial ecosystem</td>
</tr>
<tr>
<td>NF-κB</td>
<td>Nuclear factor kappa B</td>
</tr>
<tr>
<td>NOD2</td>
<td>Nucleotide-binding oligomerisation domain-containing protein 2</td>
</tr>
<tr>
<td>NSP</td>
<td>Non-starch polysaccharide</td>
</tr>
<tr>
<td>rRNA</td>
<td>Ribosomal ribonucleic acid</td>
</tr>
<tr>
<td>P80</td>
<td>Polysorbate-80</td>
</tr>
<tr>
<td>PGA</td>
<td>Physician global assessment</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>qPCR</td>
<td>Quantitative polymerase chain reaction</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomised controlled trial</td>
</tr>
<tr>
<td>RS</td>
<td>Resistant starch</td>
</tr>
<tr>
<td>SCCAI</td>
<td>Simple clinical colitis activity index</td>
</tr>
<tr>
<td>SCD</td>
<td>Specific carbohydrate diet</td>
</tr>
<tr>
<td>SCFA</td>
<td>Short-chain fatty acids</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-like receptor</td>
</tr>
<tr>
<td>TNFα</td>
<td>Tumour-necrosis factor alpha</td>
</tr>
<tr>
<td>TRPV1</td>
<td>Transient receptor potential vanilloid type 1</td>
</tr>
<tr>
<td>UC</td>
<td>Ulcerative colitis</td>
</tr>
<tr>
<td>VOC</td>
<td>Volatile organic compounds</td>
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<tr>
<td>5-ASA</td>
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1 Introduction
1.1 Inflammatory bowel disease

1.1.1 Introduction

Inflammatory bowel disease (IBD) includes Crohn’s disease (CD), characterised by discontinuous transmural inflammation that can affect all regions of the gastrointestinal (GI) tract and ulcerative colitis (UC), characterised by continuous mucosal inflammation that is isolated to the colon beginning in the rectum and extending to varying degrees. Patients with IBD generally experience periods of remission (i.e. absence of objective evidence of GI inflammation), interspersed with periods of active inflammation. Symptoms of abdominal pain, diarrhoea, anaemia and weight loss are characteristic of active CD. Transmural inflammation can result in complications such as fistulas between one part of the GI tract and another part of the GI tract; other organs; or the skin. Intestinal obstruction can also occur as a result of strictures and may necessitate intestinal resections. Hallmark symptoms of active UC are rectal bleeding, diarrhoea and urgency, and many patients also experience abdominal pain.

Inflammatory bowel disease represents a significant individual and societal burden since it typically presents at a young age and a lifetime of medical management is required. Despite medical management, many patients continue to live with considerable GI symptoms and disability, which can result in loss of education and difficulty attaining employment. Inflammation can also lead to growth failure and delayed puberty in young people. Lifetime costs of IBD are comparable to other chronic diseases and indirect costs on society may exceed direct costs of medical management, although with the increasing use of biologic therapies the contribution of medical management to the overall financial burden of IBD are likely to increase (Mowat et al., 2011, Luces and Bodger, 2006).

1.1.2 Prevalence and incidence

Traditionally, IBD was considered a disease of the economically-developed world. However, rising incidence in areas that were previously of low incidence, such as Asia, South America and Southern and Eastern Europe suggests that the classic geographic distribution of IBD is changing (Burisch and Munkholm, 2013, Ananthakrishnan, 2015). Worldwide IBD incidence and prevalence are increasing with time, with the highest annual incidence in Europe and North America, reported at 12.7 and 20.2 cases per 100,000 person-years for CD and 24.3 and 19.2 cases per 100,000 person-years for UC (Molodecky et al., 2012). The prevalence of IBD in Europe is estimated at 322 per 100,000 for CD and 505 per 100,000 persons for UC (Molodecky et al., 2012). An East-West gradient of IBD incidence was established in an inception cohort of 1515 European patients, with greater incidence rates in Western than Eastern Europe (Burisch et al., 2014a). The reasons for this remain unclear but may relate to different environmental exposures.
in Western Europe. Furthermore, an early European study (Economou and Pappas, 2008) and a more recent North American study (Schultz and Butt, 2012) observed a north-south gradient in IBD incidence, hypothesised to relate to vitamin D status.

### 1.1.3 Pathogenesis of IBD

The pathogenesis of IBD remains elusive, however it is likely to arise as a result of a combination of genetics, GI microbiota, dysregulated immune function and environmental exposures including smoking, diet and antibiotics (Figure 1.1) (Ananthakrishnan, 2015). The generally accepted notion is that IBD results from an inappropriate and uncontrolled inflammatory response to the GI microbiota in a genetically susceptible host (Khor et al., 2011). In this section, the major factors hypothesised to be involved in IBD pathogenesis are discussed.

![Factors potentially involved in the pathogenesis of IBD](image)

**Figure 1.1** Factors potentially involved in the pathogenesis of IBD. Green boxes: environmental factors potentially involved in IBD pathogenesis include vitamin D exposure, smoking, hygiene, diet, medications and appendectomy; blue box: stress is hypothesised to play a role in the risk of IBD and IBD relapse through the brain-gut axis; red box: some individuals with IBD possess single-nucleotide polymorphisms associated with susceptibility to IBD; yellow box: an aberrant microbiome composition or function may play a role in IBD development.

#### 1.1.3.1 Genetics

Familial aggregation studies and genome-wide association studies (GWAS) support the role of genetic predisposition in the development of IBD. However, IBD-associated loci explain only 13.1% and 8.2% of the variance in CD and UC risk respectively, suggesting that other risk factors
Chapter 1: Introduction

play a predominant role (Liu et al., 2015). In line with these findings, only 2-14% of patients with CD and 8-14% of patients with UC report a family history of IBD and the risk of a first-degree relative developing IBD is 5% with a CD proband and 1.6% for a UC proband (Ananthakrishnan, 2015). Twin studies have shed further insight into the genetic basis of IBD, with relatively high CD concordance of 20-50% in monozygotic twins. This is considerable but not 100% concordance, thus further supporting the involvement of other risk factors in IBD development.

To date, GWAS including individuals of European and non-European descent have identified 200 IBD risk loci, many of which encode genes relating to intestinal barrier function, microbial defence, innate immune regulation, reactive oxygen species generation, autophagy and regulation of adaptive immunity (Liu et al., 2015). Some are protective and some predisposing alleles, and most are shared between CD and UC, with some that are specific to one or the other. The first genetic locus to be associated with CD risk was that of NOD2 (also known as caspase recruitment domain-containing protein 15 or CARD15), with three variants associated with greater risk (Hugot et al., 2001). This is an intracellular protein found in antigen-presenting cells and Paneth cells, bearing a leucine-rich repeat domain responsible for the recognition of the microbial ligand, muramyl dipeptide. The NOD domain is crucial in the activation of the protein following ligand recognition and the downstream activation of the pro-inflammatory transcription factor NF-κB (nuclear factor kappa B) (Strober et al., 2007). Impaired activation of pro-inflammatory NF-κB with NOD2 mutations should presumably reduce inflammation, but clearly the opposite is observed. This is postulated to relate to an impaired host defence and greater bacterial colonisation of the mucosa. Alternatively, NOD2 may in fact downregulate NF-κB. Interestingly, mice with NOD2 mutations do not develop spontaneous colitis until exposed to an antigen recognised by a large proportion of T-cells, suggesting bacterial involvement (Watanabe et al., 2006). Some IBD risk loci are involved in common immunological pathways, for example ATG16L1, NOD2, IRGM and LRRK2 all play a role in autophagy, indicating a role for dysregulated immune function in IBD risk.

1.1.3.2 Gastrointestinal microbiota

The GI microbiota plays numerous roles important to the human host, including the inhibition of pathogen colonisation, the digestion of substrates that are inaccessible to humans, and the priming and development of the immune system. The colonic microbiota consists of $10^{11}$ or $10^{12}$ bacterial cells per gram of luminal contents, representing the most densely populated region of the GI tract (Sender et al., 2016).

There are several lines of evidence suggesting a role for the GI microbiota in IBD pathogenesis. Firstly, as discussed above, many of the genetic loci associated with IBD risk relate to pathways
involved in microbial defence. Secondly, studies indicate a lower risk of IBD associated with larger family size, early life exposure to animals, and breastfeeding, exposures that could modify the GI microbiota (Ng et al., 2015, Timm et al., 2014, Xu et al., 2017). Thirdly, germ-free mice fail to spontaneously develop colitis and therefore the microbiota are assumed to be required for IBD development (Sartor and Mazmanian, 2012). Fourthly, restoration of the faecal stream after reversal of a diverting ileostomy results in CD recurrence, implicating a role for the luminal contents in disease initiation (Rutgeerts et al., 1991). Finally, a systematic review and meta-analysis concluded that antibiotic therapy is effective for inducing remission in IBD, suggesting that the GI microbiota may have some role in GI inflammation (Khan et al., 2011).

The altered microbiota consistently observed in IBD provides further evidence of a potential role of the microbiome in IBD pathogenesis. This so-called ‘dysbiosis’ is characterised by reduced microbial diversity, particularly within the Firmicutes phylum, which is generally more marked in CD than in UC. Reduced Firmicutes and increased Proteobacteria are commonly observed in CD, while in UC reduced Roseburia and Faecalibacterium prausnitzii have been observed (Machiels et al., 2013) (Table 1.1). The microbiome may vary according to disease activity, with lower diversity in inflamed compared to non-inflamed tissue in CD (Sepehri et al., 2007, Walker et al., 2011). Adherent invasive Escherichia coli is enriched in ileal CD particularly, and this may relate to its preference for and ability to thrive in an inflammatory environment (Rolhion and Darfeuille-Michaud, 2007). Recent studies have utilised 16S rRNA sequencing to establish that the IBD microbiome displays greater temporal instability than that of healthy controls and that a CD-associated microbial signature exists (Halfvarson et al., 2017, Pascal et al., 2017).

The bacterium most consistently shown to be reduced in IBD, particularly in ileal CD, is F. prausnitzii of the Clostridiaceae family (Table 1.1). This is a strictly anaerobic butyrate-producing bacterium that resides in the colon and represents around 5% of the faecal microbiota (Hold et al., 2003). The growth of F. prausnitzii in the colon is influenced by substrate availability, pH, oxygen levels and bile acid concentrations, and the study of F. prausnitzii has been hindered by its extreme oxygen-sensitivity (Lopez-Siles et al., 2017). In vitro and in vivo (TNBS and DNBS colitis models) studies have demonstrated anti-inflammatory effects of F. prausnitzii with the release of metabolites capable of blocking NF-κB activation and pro-inflammatory IL-8 production (Martin et al., 2014, Rossi et al., 2016, Sokol et al., 2008, Sokol et al., 2009). A protein secreted by F. prausnitzii, termed microbial anti-inflammatory molecule, has been isolated and identified as responsible for its anti-inflammatory effects (Quévrain et al., 2015). Strain specificity in the immunomodulatory properties of F. prausnitzii has recently been identified (Martín et al., 2017).
There may be functional consequences of GI microbiome aberrations in IBD. In gnotobiotic mice harbouring only *F. prausnitzii* and *E. coli*, a metabolic signature was associated with the anti-inflammatory effects of *F. prausnitzii* (Miquel et al., 2015). A lower *F. prausnitzii* abundance at the point of intestinal resection is associated with greater risk of post-operative recurrence at 6 months in patients with CD (Sokol et al., 2008). Furthermore, a study of the mucosa-associated microbiota in patients with CD at the point of intestinal resection and at recurrence revealed that patients with post-operative recurrence at 6 months exhibited a microbiota with greater proteolytic fermentation capacity, while those remaining in remission retained saccharolytic genera including *Bacteroides*, *Prevotella* and butyrate-producing *Firmicutes* that may include *F. prausnitzii* (De Cruz et al., 2015).

Although numerous studies have assessed the GI microbiome in patients with established IBD (Table 1.1), they are limited by small sample sizes, an assessment of only the luminal microbiota, variation in the patients recruited (CD vs. UC; active vs. inactive; surgery vs. no surgery) and the method used to characterise the microbiota. In addition, case-control studies are inherently confounded by the effect of prolonged inflammation and medication on the GI microbiota, posing the question as to whether microbiota alterations are a cause or consequence of IBD. A large study of the luminal and mucosal GI microbiota of 1,742 newly diagnosed, treatment naïve paediatric and adult patients with CD attempted to address some of these limitations (Gevers et al., 2014). Furthermore, an analysis of siblings of CD probands observed a dysbiosis mirroring that of CD, characterised by reduced *F. prausnitzii*, suggesting that dysbiosis may precede CD development (Hedin et al., 2014, Hedin et al., 2016).
<table>
<thead>
<tr>
<th>Patients</th>
<th>Sample site</th>
<th>Method of bacterial analysis</th>
<th>Species or genera increased in IBD</th>
<th>Species or genera decreased in IBD</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal microbiota</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 CD (9 inactive, 8 active) 16 healthy controls</td>
<td>Faeces</td>
<td>Quantitative dot blot hybridisation</td>
<td>Enterobacteria</td>
<td>Clostridium coccoides</td>
<td>(Sekis et al., 2003)</td>
</tr>
<tr>
<td>6 CD</td>
<td>Faeces</td>
<td>Microarray</td>
<td>Bifidobacterium (active CD)</td>
<td>Clostridium leptum</td>
<td>(Manichanh et al., 2006)</td>
</tr>
<tr>
<td>13 CD 13 UC 13 healthy controls</td>
<td>Faeces</td>
<td>FISH</td>
<td></td>
<td>Clostridium coccoides (UC)</td>
<td>(Sokol et al., 2006)</td>
</tr>
<tr>
<td>13 CD 13 UC 13 healthy controls</td>
<td>Faeces</td>
<td>Microarray and qPCR</td>
<td>Enterococcus spp.</td>
<td>Faecalibacterium prausnitzii</td>
<td></td>
</tr>
<tr>
<td>127 UC 87 healthy controls 116 UC 29 first-degree relatives 31 healthy controls</td>
<td>Faeces</td>
<td>Denaturing gradient gel electrophoresis</td>
<td></td>
<td>Roseburia hominis</td>
<td>(Machiels et al., 2013)</td>
</tr>
<tr>
<td>10 inactive CD 10 healthy controls</td>
<td>Faeces</td>
<td>16S rRNA sequencing</td>
<td>Actinomyces Bifidobacteria</td>
<td>Blautia faecis</td>
<td>(Takahashi et al., 2016)</td>
</tr>
<tr>
<td>Patients</td>
<td>Sample site</td>
<td>Method of bacterial analysis</td>
<td>Species or genera increased in IBD</td>
<td>Species or genera decreased in IBD</td>
<td>Reference</td>
</tr>
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<td>------------------------------------------------</td>
</tr>
<tr>
<td>34 CD</td>
<td>Faeces</td>
<td>16S rRNA sequencing</td>
<td>Streptococcus (UC)</td>
<td>Faecalibacterium (CD)</td>
<td>(Pascal et al., 2017)</td>
</tr>
<tr>
<td>33 UC</td>
<td></td>
<td></td>
<td></td>
<td>Christensenellaceae (CD)</td>
<td></td>
</tr>
<tr>
<td>40 healthy controls</td>
<td></td>
<td></td>
<td></td>
<td>Methanobrevibacter (CD)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Oscillospira (CD)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Sutterella (UC)</td>
<td></td>
</tr>
<tr>
<td>49 CD (1-10 samples each)</td>
<td>Faeces</td>
<td>16S rRNA sequencing</td>
<td>Alistipes massiliensis (UC)</td>
<td>Faecalibacterium prausnitzii</td>
<td>(Halfvarson et al., 2017)</td>
</tr>
<tr>
<td>60 UC (1-10 samples each)</td>
<td></td>
<td></td>
<td></td>
<td>Ruminococcaceae (ileal CD)</td>
<td></td>
</tr>
<tr>
<td>9 healthy controls (1-10 samples each)</td>
<td></td>
<td></td>
<td></td>
<td>Lachnospiraceae (ileal CD)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Clostridiales (ileal CD)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Prevotella copri (ileal CD)</td>
<td></td>
</tr>
<tr>
<td>49 CD</td>
<td>Faeces</td>
<td>16S rRNA sequencing</td>
<td></td>
<td>Escherichia fergusonii</td>
<td>(Hedin et al., 2016)</td>
</tr>
<tr>
<td>6 UC</td>
<td>Mucosa</td>
<td>16S rRNA sequencing</td>
<td>Enterobacteriaceae (CD)</td>
<td>Faecalibacterium prausnitzii</td>
<td>(Walker et al., 2011)</td>
</tr>
<tr>
<td>5 healthy controls</td>
<td>Mucosa</td>
<td>qPCR</td>
<td>Escherichia coli (CD)</td>
<td>Faecalibacterium prausnitzii</td>
<td>(Lopez-Siles et al., 2014)</td>
</tr>
<tr>
<td>21 inactive CD</td>
<td>Mucosa</td>
<td>16S rRNA sequencing</td>
<td>Escherichia fergusonii</td>
<td>Faecalibacterium prausnitzii</td>
<td></td>
</tr>
<tr>
<td>17 healthy siblings</td>
<td>Mucosa</td>
<td>qPCR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19 healthy controls</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Luminal and mucosal microbiota</td>
<td>Faeces</td>
<td>16S rRNA sequencing</td>
<td>Escherichia (CD)</td>
<td></td>
<td>(Morgan et al., 2012)</td>
</tr>
<tr>
<td>121 CD</td>
<td>Mucosa</td>
<td></td>
<td>Shigella (CD)</td>
<td>Roseburia</td>
<td></td>
</tr>
<tr>
<td>75 UC</td>
<td></td>
<td></td>
<td>Phascolarctobacterium</td>
<td>Ruminococcaceae (CD)</td>
<td></td>
</tr>
<tr>
<td>27 healthy controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21/15 CD</td>
<td>Faeces</td>
<td>qPCR</td>
<td>Bifidobacteria (active UC mucosa)</td>
<td>Faecalibacterium prausnitzii</td>
<td>(Wang et al., 2014)</td>
</tr>
<tr>
<td>34/29 UC</td>
<td>Mucosa</td>
<td></td>
<td>Lactobacillus (active CD mucosa)</td>
<td>(all IBD, faecal and mucosal)</td>
<td></td>
</tr>
<tr>
<td>21 healthy controls</td>
<td></td>
<td></td>
<td>Escherichia coli</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1.1.3.3 **Intestinal permeability**

The mucosal GI barrier is composed of the epithelial layer, including a web of tight junction proteins, and the overlying mucus, that selectively permits passage of nutrients while excluding the majority of the luminal contents. One theory for IBD pathogenesis is that an impaired GI mucosal barrier results in bacterial translocation and immune activation with a subsequent inflammatory cascade. Reduced expression of tight junction proteins, including occludins, claudins and junctional adhesion molecules and altered structure of tight junctions have been shown in IBD (Schmitz et al., 1999) (Zeissig et al., 2007). However, altered permeability in the presence of active GI inflammation is likely an effect of tumour necrosis factor alpha (TNFα) and other cytokines involved in the inflammatory cascade on tight junction protein expression and epithelial apoptosis (Marini et al., 2003). That said, impaired intestinal permeability has been shown to precede inflammation in murine models of chemically-induced colitis. Furthermore, murine models with genetically impaired GI barrier function spontaneously develop inflammation (Pastorelli et al., 2013). However, this is not consistent among all animal models with impaired tight junctions, suggesting that other factors are involved in initiating an inflammation. Furthermore, animal colitis models do not perfectly replicate human IBD. In support of increased GI permeability as a causative factor in human IBD, increased GI permeability has been observed in siblings of IBD probands (Hollander et al., 1986), although this is inconsistent between studies (Hedin et al., 2014). A greater likelihood of CD relapse was observed in patients with increased GI permeability (Arnott et al., 2000). However, this was in a small number of patients and it remains unclear whether impaired intestinal permeability precedes human IBD or is simply a consequence of elevated inflammatory cytokines during inflammation.

1.1.3.4 **Immune dysregulation**

Chronic inflammation in IBD relates to a dysregulated immune response and therefore the immune system has been thoroughly investigated as a major factor in the pathogenesis of IBD. With the classical view that IBD is T-cell mediated, adaptive immunity has been the focus of this research. Accumulation of T helper 1 and T helper 17 cells (expressing cytokines including IFN-γ, IL-17A and IL-22) is observed in IBD, and some investigators have suggested an accumulation of T helper 2 cells in UC specifically (Fuss et al., 1996). There is increasing interest in the role of T regulatory cells, which under normal circumstances would suppress inflammation, a function that appears defective in IBD. The T regulatory cell response may be insufficient to suppress inflammation in IBD, which could relate to intrinsic cellular defects or a result of the extrinsic cytokine milieu in IBD. Furthermore, T regulatory cells are influenced by the GI microbiota,
forging a link between microbiota aberrations and immune suppressive function in IBD (Mayne and Williams, 2013).

Although evidence to date has focussed on the role of adaptive immunity in IBD, emerging evidence suggests a role of the innate immune system in IBD. This includes an altered GI mucosal barrier, antimicrobial peptide production, and dysregulated innate lymphoid cell (ILC) function (de Souza and Fiocchi, 2016). The latter innate lymphocytes are found in mucosal tissues and produce IBD-specific cytokines but lack the antigen-specific receptors found on T and B-cells. In animal models of IBD, ILC’s have been implicated in GI inflammation. Furthermore, altered numbers of ILC’s have been observed in IBD (Goldberg et al., 2015). Research in this field will likely focus on ILC’s as therapeutic targets in IBD.

Dendritic cells (DC) are innate antigen-presenting cells, capable of sampling GI antigen by extending dendrites into the lumen. Upon presentation of antigen by intestinal DC to naïve T-cells in mesenteric lymph nodes and Peyer’s patches, T-cells differentiate into either a regulatory or effector phenotype. In IBD mucosa, DC express increased levels of TLR-2, TLR-4, IL-12 and IL-6 compared to controls, compatible with an enhanced ability to sample microbial antigen and being in an activated state (Hart et al., 2005). Given that DC phenotype and cytokine production has a role in the subsequent T-cell phenotype, an altered DC phenotype could play a role in IBD pathogenesis (Stagg et al., 2003).

Leukocytes must traffic to the appropriate tissues of the body in order to carry out the required effects. Naïve T-cells express receptors permitting entry to lymphoid organs but are usually excluded from peripheral tissues such as the GI tract. In contrast, antigen-experienced effector T-cells acquire expression of molecules allowing migration into tissues. The major homing molecule required for T-cell entry to the GI tract is the integrin α4β7 (Berlin et al., 1993). Interaction with its ligand, mucosal addressin cell adhesion molecule-1 (MAdCAM-1) on intestinal high endothelial venules facilitates T-cell entry into the GI tract. T-cells activated by DC in the gut-associated lymphoid tissue acquire a gut-homing phenotype, suggesting a role of DC or the environment in inducing this phenotype. Retinoic acid appears crucial in inducing a gut-homing phenotype in T-cells and in contrast to DC from other tissues, intestinal DC (likely specifically CD103+ DC) express retinol dehydrogenases required for retinoic acid generation (Iwata et al., 2004). In IBD, there is an abnormal and persisting infiltration of T-cells into the GI mucosa. Upregulation of MAdCAM-1 on high endothelial venules in animal models of colitis and human IBD and redistribution of gut-homing T-cells from the circulation to the GI mucosa in IBD suggests a role of dysregulated T-cell gut homing in IBD pathogenesis (Hart et al., 2004a, Hart et al., 2004b).
1.1.3.5 Environmental factors

Numerous environmental exposures have been associated with the risk of developing IBD, including smoking, appendectomy, GI infection, antibiotic use, diet (which will be discussed in section 1.2), non-steroidal anti-inflammatory use, oral contraceptives, stress, physical activity and sleep. None of these exposures appear sufficient to induce IBD in isolation but may interact with other risk factors such as genetic predisposition and immune dysregulation to increase IBD risk (Ananthakrishnan, 2015). Some of these environmental exposures will be discussed below.

Smoking has long been identified as a factor associated with IBD risk. A meta-analysis revealed a twofold increased risk of CD associated with current and previous smoking, while current smoking was inversely associated with UC risk (Mahid et al., 2006). Continued smoking is also associated with a worse prognosis and more complex disease in CD, while in UC smoking cessation can be associated with relapse (To et al., 2016b, To et al., 2016a). The aetiology of the relationship between smoking and IBD risk remains unclear, however hypotheses include the effects of nitric oxide on endothelial function, a possible effect on epithelial barrier integrity, oxidative stress or the GI microbiota (Ananthakrishnan, 2015). Recently, 64 single nucleotide polymorphisms associated with IBD risk were identified as being modified by smoking status, suggesting that the impact of smoking on IBD risk may be genetically determined (Yadav et al., 2017).

Appendectomy displays a divergent association with CD and UC risk, similar to smoking, with a greater risk of CD and a lower risk of UC in patients who have had an appendectomy (Andersson et al., 2001, Andersson et al., 2003), although a reduced risk of CD once controlled for appendectomy at the point of CD diagnosis has also been demonstrated (Radford-Smith et al., 2002). Appendectomy for perforating appendicitis appears to be associated with greater risk of intestinal resection in CD, whereas appendectomy for other indications is associated with reduced CD risk (Andersson et al., 2003).

Several epidemiological and animal studies indicate a greater risk of IBD development and IBD relapse following GI infection, including Salmonella, Campylobacter, *E. coli*, *Helicobacter pylori* and *Clostridium difficile* (Schultz et al., 2017). The increased risk of IBD is greatest in the year after GI infection, suggesting this association may be partly associated with detection bias (Jess et al., 2011). This association is hypothesised to relate to lasting effects of infection on the GI microbiota, barrier function or GI immune responses to the GI microbiota (Schultz et al., 2017).

A common theory relating to IBD pathogenesis is the ‘hygiene hypothesis’, referring to the increase in autoimmune and chronic inflammatory conditions alongside an improvement in sanitation and a decrease in infectious diseases. This hypothesis suggests that a diverse GI
microbiota promotes differentiation of GI mucosal DC into tolerogenic DC, which in turn promote the development of T regulatory cells. Excessive hygiene measures and antibiotic use is proposed to limit the GI microbiota repertoire and to promote immunogenic DC, leading to effector T-cell generation and an inflammatory response (Rook, 2012). However, this hypothesis has recently come under scrutiny, and a new hypothesis has emerged suggesting that commensal environmental microbial exposures are more important in the development of the immune system than GI infections (Bloomfield et al., 2016). Greater IBD risk is associated with antibiotic use in early life in a dose-dependent fashion, at least in Western populations (Shaw et al., 2010, Kronman et al., 2012), an association hypothesised to relate to a perturbation of the developing microbiota. Interestingly, early childhood antibiotic use in Asia, where children are likely to be exposed to more GI pathogens, appears to be protective against IBD (Ng et al., 2015).

1.1.4 Diagnosis and monitoring

Diagnosis of IBD is through a combination of clinical symptoms and objective evidence from endoscopic, radiological and histologic examinations. Upon presentation, a history including recent travel, medications, smoking, family history of IBD, previous appendectomy and recent GI infections should be taken (Mowat et al., 2011). A thorough assessment of GI symptoms, physical examination and laboratory tests are important in a diagnosis of IBD. On endoscopy and histology, Crohn’s disease is characterised by the presence of focal and often granulomatous inflammation, while in UC continuous inflammation with basal plasmacytosis, crypt architectural distortion and mucosal atrophy is observed (Magro et al., 2017). Imaging, such as magnetic resonance imaging (MRI), ultrasound and computed tomography imaging may be used to assist diagnosis. This is particularly useful to examine the small intestine and for the detection of abscesses (Mowat et al., 2011). Magnetic resonance imaging is particularly useful in assessing perianal CD.

Correct classification of IBD is vital since it influences clinical decision-making, monitoring and the assessment of the risk of long-term complications. Inflammatory bowel disease phenotype can be classified using the Montreal criteria, based upon age of diagnosis, location of inflammation in the GI tract and the behaviour of disease for CD, and based upon the extent and severity of inflammation in the colon for UC (Table 1.2).
Table 1.2 The Montreal Classification of Crohn’s disease and ulcerative colitis (Satsangi et al., 2006)

<table>
<thead>
<tr>
<th>Crohn’s disease</th>
<th>Ulcerative colitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis</td>
<td>Extent</td>
</tr>
<tr>
<td>A1: &lt;16 years</td>
<td>E1: Ulcerative proctitis</td>
</tr>
<tr>
<td>A2: 17-40 years</td>
<td>E2: left-sided UC (distal)</td>
</tr>
<tr>
<td>A3: &gt;40 years</td>
<td>E3: extensive UC (pancolitis)</td>
</tr>
<tr>
<td>Location</td>
<td>Severity</td>
</tr>
<tr>
<td>L1: ileal</td>
<td>S0: clinical remission</td>
</tr>
<tr>
<td>L2: colonic</td>
<td>S1: mild UC</td>
</tr>
<tr>
<td>L3: ileocolonic</td>
<td>S2: moderate UC</td>
</tr>
<tr>
<td>L4: isolated upper disease*</td>
<td>S3: severe UC</td>
</tr>
<tr>
<td>Behaviour</td>
<td></td>
</tr>
<tr>
<td>B1: non-stricturing, non-penetrating</td>
<td></td>
</tr>
<tr>
<td>B2: stricturing</td>
<td></td>
</tr>
<tr>
<td>B3: penetrating</td>
<td></td>
</tr>
<tr>
<td>p: perianal disease modifier†</td>
<td></td>
</tr>
</tbody>
</table>

*L4 is a modifier that can be added to L1-L3 when concomitant upper GI disease is present
†"p" is added to B1-B3 when concomitant perianal disease is present

Frequency of IBD monitoring partly depends upon the course of disease and IBD medications. Patients receiving, for example, biologic therapies will be monitored regularly for clinical response and safety of the drug.

1.1.5 Impact of IBD on health-related quality of life

Chronic health conditions often impact upon health-related quality of life (HR-QOL). Inflammatory bowel disease induces significant GI symptoms and, in some cases, long-term complications of GI inflammation, which in many patients have psychosocial consequences. A large study across seven European countries revealed that patients with IBD and GI symptoms at the time of the study had poorer HR-QOL compared to the healthy population, though it is unknown whether GI symptoms related to active IBD or were functional gastrointestinal symptoms (Huppertz-Hauss et al., 2015). This study used a generic HR-QOL measure and therefore likely underestimated the impact of IBD specifically on HR-QOL. This is in contrast to IBD-specific HR-QOL measures, the most widely used being the IBD Questionnaire that encompasses issues determined to be important in IBD (Cheung et al., 2000). Characteristics identified to predict poor HR-QOL in IBD include higher perceived stress, high number of hospitalisations and relapses, low perceived support, low income, unemployment and female gender (Moradkhani et al., 2013). In another large cross-sectional study of patients with IBD, high levels of anxiety, depression and stress were associated with poor HR-QOL, suggesting that psychological parameters may influence HR-QOL (Iglesias-Rey et al., 2014).

Certain interventions have proven effective in improving HR-QOL in IBD, including drug treatments such as infliximab (Casellas et al., 2012) and non-pharmacological treatments such as exercise (Klare et al., 2015) and breathing and meditation (Gerberg et al., 2015). Since poor HR-QOL is associated with a greater number of relapses and disease severity (Graff et al., 2006),
it is likely that any treatments effective in maintaining remission and reducing inflammation would improve HR-QOL.

A systematic review identified patients with emotion-focussed and problem-focussed coping styles, the former being associated with poorer psychological outcomes and the latter associated with better psychological outcomes (McCombie et al., 2013). Likewise, a prospective analysis of patients within the first 6 months of IBD diagnosis observed both adaptive and maladaptive coping strategies, the latter of which was associated with worse outcomes (McCombie et al., 2015). These findings suggest that coping styles and strategies, which vary among patients, may have an influence on the impact of IBD on quality of life and psychological interventions targeted at manipulating coping styles may improve psychological outcomes.

1.1.6 Treatment of IBD

1.1.6.1 Medical and surgical treatment

The aims of medical management of IBD are to induce and maintain clinical and endoscopic remission, and to prevent the natural progression of the disease and the development of complications (Baumgart and Sandborn, 2012). Corticosteroids, 5-aminosalicylic acid (5-ASA), immune-suppressive drugs and biologics such as anti-TNFα antibodies are used to manage inflammation, either alone or in combination. The choice of medical therapy is multifactorial, taking into account disease activity, location and behaviour, possible side effects and previous response to medication. Within 10 years of diagnosis, 20-30% of patients with UC require surgery (Ordas et al., 2012), most commonly subtotal colectomy with a temporary ileostomy and subsequent reversal and formation of an ileo-anal pouch. In CD, multiple small intestinal and colonic resections may be required for the management of intestinal obstruction, penetration or treatment-refractory disease (Bemelman et al., 2018).

1.1.6.2 Microbial treatment

With the overwhelming evidence for a role of the GI microbiota in IBD, much research has focussed on the possible therapeutic benefit of its modification. One method of doing so is through supplementing with probiotic bacteria. Probiotics are defined as “live microorganisms which when administered in adequate amounts confer a health benefit to the host” (Hill et al., 2014), the most common being Bifidobacteria and Lactobacilli. Probiotics may confer benefit through a range of mechanisms including exclusion of pathogenic bacteria, normalisation of a perturbed microbiota, direct modulation of the GI immune system and SCFA production (Hill et al., 2014). Cellular and animal models support the potential anti-inflammatory effects of probiotics. For example, a comparative in vitro assessment of six probiotic Lactobacillus and
Bifidobacteria strains revealed that all strains influenced immune cell activation and cytokine production, however Bifidobacteria strains tended to induce greater anti-inflammatory IL-10 production (Dong et al., 2012). Furthermore, *Bifidobacterium infantis* induced a strong anti-inflammatory cytokine profile, characterised by an increase in the IL-10/IL-12 ratio, in peripheral blood mononuclear cells from older subjects (You and Yaqoob, 2012). Lactobacilli and Bifidobacteria modulate DC cytokine production *in vitro* (You et al., 2014). In line with these findings, probiotic treatment in patients with UC increased mucosal DC IL-10 and reduced IL-12p40 production, suggesting that anti-inflammatory effects of probiotics may be mediated by DC (Ng et al., 2010). *In vivo*, a mixture of Bifidobacterium and Lactobacillus species reduced the severity of dextran sodium sulphate (DSS) colitis (Nanda Kumar et al., 2008).

The most conclusive evidence for probiotics in IBD is for a combination probiotic containing *Bifidobacterium* and *Lactobacillus* species (VSL#3®) in maintaining remission in pouchitis (Singh et al., 2015). In contrast, there is no evidence supporting the use of probiotics in CD, but in UC there appears to be some benefit in the induction and maintenance of remission. A recent systematic review and meta-analysis concluded that VSL#3 was more effective in inducing remission in UC compared to placebo, and equally effective compared to 5-ASA for maintenance of remission (Derwa et al., 2017). The effectiveness of probiotics in UC but not CD may be explained by differences in baseline microbiota, different disease pathogenesis and greater heterogeneity in terms of CD location and behaviour.

The GI microbiota may also be altered by supplementation with prebiotic carbohydrates. The definition of a prebiotic has recently been updated by consensus and is now defined as “a substrate that is selectively utilised by host microorganisms conferring a health benefit” (Gibson et al., 2017). Fructans and galacto-oligosaccharides are the only carbohydrates that have been consistently shown to meet prebiotic criteria. Prebiotics likely exert effects on the host through increasing the abundance of GI bacteria with putative benefits to host health, which has been substantiated in healthy individuals (Ramirez-Farias et al., 2009, Ramnani et al., 2015, Lomax et al., 2012, Vandeputte et al., 2017). However, prebiotic effects may also be mediated through SCFA production or direct ligation of pattern recognition receptors on immune cells, although there is currently sparse evidence for the latter mechanism (Roberfroid et al., 2010). Traditionally, prebiotics were assumed to predominantly stimulate the growth of Bifidobacteria and Lactobacilli, however molecular methods of microbiota characterisation have revealed that many other species utilise prebiotic substrates or may be increased due to cross-feeding reactions (Moens et al., 2016).

In terms of the clinical effectiveness of prebiotics in IBD, a large placebo-controlled trial showed no effect of fructan supplementation on disease activity or GI microbiota composition.
(measured using FISH) in active CD, although analysis using a more comprehensive technique such as sequencing could have revealed bacterial alterations (Benjamin et al., 2011b). An RCT of patients with active UC revealed similar effects of fructan supplementation and placebo on disease activity, although faecal calprotectin significantly reduced in the fructan group only (Casellas et al., 2007). Supplementation was only for a duration of two weeks and the trial had a small sample size and failed to monitor the effect of fructan supplementation on the GI microbiota. Thus, the current evidence suggests that prebiotics are unlikely to be effective in the treatment of active IBD. However, the role of prebiotic supplementation in maintaining remission remains unexplored.

Finally, in recent years there has been considerable interest in the transfer of GI microbiota from a healthy donor to a recipient with a perturbed microbiota, termed faecal microbiota transplant (FMT). For the management of Clostridium difficile infection, FMT is extremely effective and is now part of clinical guidelines as an approved therapy. However, the current evidence for FMT in IBD is less conclusive. A systematic review and meta-analysis identified 14 cohort studies and four RCTs of FMT in active UC, and revealed a remission rate of 28% following FMT compared to 9% following placebo in the RCTs (Costello et al., 2017). Although this is promising data, there are numerous questions that remain unanswered, including the optimal dosing regimen, route of administration and long-term efficacy and safety of FMT in UC. Furthermore, the choice of donor may have an influence on response to FMT, with some donors inducing remission in a greater proportion of patients (Moayyedi et al., 2015). A meta-analysis revealed that FMT may be effective in CD, however the included studies in CD were uncontrolled and RCTs are needed to confirm the findings (Colman and Rubin, 2014).

1.1.6.3 Dietary treatment

Undoubtedly the most widely used dietary treatment for IBD is exclusive enteral nutrition (EEN), in which patients obtain their nutritional requirements from liquid formula administered either orally or via a feeding tube. Interest in EEN as primary therapy for CD emerged following studies showing an improvement in IBD activity in pre-surgical patients given enteral formula as nutrition support (Voitk et al., 1973).

Trials of EEN in CD have, justifiably, lacked a placebo control arm, since it is extremely difficult to design a placebo treatment that mimics the appearance and flavour of EEN. Further limitations of these trials include the high withdrawal rates (particularly in trials of orally administered EEN), inclusion of patients with heterogeneous disease duration, location and severity, varied EEN duration and a lack of blinding, even in trials comparing different types of formula where blinding could realistically be employed. A systematic review and meta-analysis
established no differences in response to EEN according to fat composition (type or quantity) or protein source (elemental, semi-elemental or polymeric formula) (Narula et al., 2018). In the same meta-analysis, EEN was less effective than corticosteroids for inducing remission in active CD, however several of the trials combined corticosteroids with other medication such as 5-ASA. Furthermore, most of the studies employed an intention-to-treat analysis. In the context of a high withdrawal rate this does not adequately reflect the effectiveness of the therapy when followed optimally. In clinical practice, EEN is generally recommended only to patients likely to be able to adhere. As expected, the per-protocol analysis of the aforementioned meta-analysis revealed no significant difference between EEN and corticosteroids (Narula et al., 2018).

Mechanisms of EEN in CD are hypothesised to include an effect on the GI microbiota, with studies indicating drastic bacterial alterations following EEN (Kaakoush et al., 2015, Quince et al., 2015), including a paradoxical decline in anti-inflammatory *F. prausnitzii*. Other mechanisms might include direct anti-inflammatory effects, reduction in food-derived antigens and delivery of essential nutrients to the GI mucosa (Lee et al., 2015).

A meta-analysis revealed EEN and corticosteroids to have equal effectiveness in inducing remission of active CD in children (Swaminath et al., 2017). However, EEN remains first-line therapy for CD in children, in whom corticosteroids are contraindicated (Ruemmele et al., 2014). In adults with active CD, EEN may be used in patients wishing to avoid medical treatments or in whom corticosteroids are contraindicated, or as a bridge to other treatments. There is evidence that EEN may enhance post-surgical outcomes when administered prior to surgery (Wang et al., 2016, Li et al., 2014a) and that it may enhance mucosal healing, which is associated with better long-term outcomes (Grover et al., 2016, Berni Canani et al., 2006).

In one study of adults and children with active CD, combining partial enteral nutrition (providing 50% of energy requirements) with a diet excluding foods hypothesised to alter the GI microbiota or intestinal permeability resulted in 62% of participants entering remission (Sigall Boneh et al., 2017). This trial suggests that diets combining enteral nutrition and exclusion diets may have a role in CD management, although this was an uncontrolled study and requires confirmation in controlled trials.

The Specific Carbohydrate Diet (SCD) manipulates dietary carbohydrates with the proposed purpose of modifying the GI microbiota, permitting only monosaccharides and excluding disaccharides and polysaccharides. The SCD was initially developed for the management of coeliac disease prior to the development of a gluten-free diet. It has since gained popularity for the management of IBD, although evidence for the effectiveness of this diet is limited to small retrospective and prospective uncontrolled studies (Obih et al., 2016, Suskind et al., 2014, Cohen
et al., 2014). Randomised controlled trial evidence of the effectiveness and safety of the SCD is required before widespread use in clinical practice.

Recently, interest in so-called 'anti-inflammatory' diets, that increase and decrease putative anti- and pro-inflammatory dietary components, has emerged in IBD. In a recent prospective uncontrolled study, improvements in clinical and endoscopic disease activity were observed following one such 'anti-inflammatory' diet, however without a control group the effectiveness of the diet remains to be established (Konijeti et al., 2017). Another ‘anti-inflammatory’ diet had no impact on relapse rates in patients with quiescent UC compared to Canadian national healthy eating guidelines but faecal calprotectin was maintained in the anti-inflammatory diet group (Keshteli et al.). However, to date this has been published only in abstract form, and a major limitation is the likely overlap between the dietary interventions. Furthermore, the proposed mechanisms and justification for the inclusion and restriction of certain dietary components in the dietary protocols are unclear, and the impact of these diets on nutritional adequacy is a major concern in the context of IBD. Whole diet interventions aiming to reduce intestinal inflammation warrant further investigation.

1.2 Diet and nutritional intake in IBD

A range of dietary components and patterns have been associated with the development of IBD and the risk of relapse, although no clear evidence exists that indicates the restriction of a particular foodstuff would prevent the onset or relapse of IBD (Hou et al., 2011). Numerous studies indicate that patients with IBD consider diet an important factor in the initiation of IBD relapse and GI symptoms in general, as well as reporting difficulties maintaining body weight and enjoying food and mealtimes (Triggs et al., 2010, Prince et al., 2011, Zallot et al., 2013, Limdi et al., 2016). Patients with IBD are at heightened risk of malnutrition, the cause of which is multifactorial. Furthermore, treatment for IBD can involve dietary manipulation, namely exclusive enteral nutrition (EEN), which was discussed in section 1.1.6.3. The role of diet in the pathogenesis and perpetuation of IBD, and nutritional concern in IBD, will be discussed in this section.

1.2.1 Diet in IBD pathogenesis

Several dietary components have been associated with IBD risk. These include dietary patterns characteristic of a 'Western' diet including high sugar and fat intake and low fruit and vegetable intake (Hou et al., 2011). Prospective cohort studies reveal an inverse association between fibre intake and CD risk (Ananthakrishnan et al., 2013, Amre et al., 2007), which could relate to the effects of fibre on the GI microbiota and SCFA production or direct immunological effects. Several studies, including those prospectively assessing dietary intake, reveal a greater IBD risk
with higher total protein and meat consumption, although this association is not consistent (Jantchou et al., 2010). In addition to assessing the risk of developing IBD associated with dietary components, some studies have investigated dietary factors associated with risk of relapse. For example, a recent prospective evaluation revealed that patients with CD in the highest quartile of fibre intake were less likely to relapse, although disease activity was measured using symptom-based, non-objective disease activity indices and dietary assessment was limited to a food frequency questionnaire (Brotherton et al., 2016). High intakes of red and processed meat, protein, alcoholic beverages, sulphur and sulphates have also been associated with greater risk of UC relapse (Jowett et al., 2004a). Observational studies assessing the role of dietary components and patterns in IBD risk carry methodological limitations, including but not limited to the measurement of dietary intake (food frequency questionnaires rather than more robust food records), recall bias associated with assessing pre-diagnosis dietary intake following a diagnosis of IBD, and possible reverse causality associated with assessing dietary patterns directly preceding diagnosis. Difficulties in interpreting the results of epidemiological studies of dietary risk factors arise from the complexity of foods and the co-existence of nutrients.

Vitamin D has an established role in calcium absorption and bone mineralisation and is recommended for the prevention and treatment of osteoporosis in IBD (Scott et al., 2000). However, the vitamin D receptor is expressed by cells of at least thirty different tissues in the body, including intestinal tissue and immune cells. Emerging evidence from epidemiological, in vitro, in vivo and several human intervention studies indicate a potential role for vitamin D in IBD. Vitamin D deficiency is common in IBD and poor vitamin D status is associated with greater disease activity, however it is impossible to establish a causal relationship in the context of established disease, where reduced intestinal absorption and poor sunlight exposure may play a role in suboptimal vitamin D levels (Suibhne et al., 2012, O'Sullivan, 2015). The geographical distribution of IBD prevalence and the observed north-south gradient is reflective of vitamin D status. Furthermore, a lower risk of CD was estimated in women with a higher predicted vitamin D status in an analysis of the prospective Nurses’ Health Studies, although caution must be exerted in drawing conclusions from predicted vitamin D status (Ananthakrishnan et al., 2012). Three RCTs have investigated vitamin D supplementation in CD. Two of these RCTs demonstrated no effect of vitamin D₃ supplementation on IBD activity (Jorgensen et al., 2010, Raftery et al., 2015), and the third was a pilot study (Narula et al., 2017). These trials vary in duration, vitamin D dose, and outcomes, and do not provide convincing evidence for vitamin D as an anti-inflammatory therapy.

Natural and synthetic emulsifying agents are added to foods during manufacturing to stabilise water and lipid mixtures. A strong positive correlation is observed between emulsifier
consumption and CD incidence, leading to increased interest in emulsifier consumption as a possible factor in CD pathogenesis (Roberts et al., 2013). Emulsifiers are present in ice-creams and mayonnaise. Major emulsifying agents include lecithin, derived from egg whites, mono- and diglycerides, carrageenan and the synthetic compounds polysorbate-80 (P80) and carboxymethylcellulose (CMC).

In vitro, P80 increases *E. coli* translocation across epithelia, in contrast to plantain and broccoli fibre which reduces translocation (Roberts et al., 2010). Furthermore, P80 and CMC result in greater bacterial epithelial encroachment in mice, reduced mucus thickness, greater numbers of mucosa-adherent bacteria and altered microbiota composition, the latter of which is characterised by reduced Bacteroidales and increased mucolytic *Ruminococcus gnavus* (Chassaing et al., 2015). Interestingly, emulsifier treatment led to subtle inflammation in wild-type mice while overt colitis was observed in mice with a genetic immune defect (IL-10 or TLR5 ablation), suggesting a potential genetic predisposition to inflammation following emulsifier consumption. In a subsequent study utilising the mucosal simulator of the human intestinal microbial ecosystem (M-SHIME) model, emulsifier treatment increased bioactive flagella expression and this was replicated when the microbiota from CMC-treated mice was transferred into germ-free mice, indicating that emulsifiers may induce a more inflammatory microbiota (Chassaing et al., 2017). According to these landmark studies, proposed mechanisms of emulsifiers in the pathogenesis of CD include enhanced bacterial translocation and immune stimulation and an increase in the inflammatory potential of the GI microbiota, potentially on the background of genetic immune defects. The effect of emulsifiers on GI inflammation in humans is yet to be elucidated. Furthermore, whether emulsifier restriction could represent an effective therapeutic option for IBD remains to be investigated.

1.2.2 Nutritional status and concerns in IBD

Given that IBD is a disease of the GI tract, the site of digestion, it is logical to expect that diet and nutrition will be in some way impacted by the disease, and in particular CD where active disease can occur anywhere in the GI tract. As expected, patients with IBD are more at risk of protein energy malnutrition and specific nutrient deficiencies than the general population. This is particularly prevalent in patients with active IBD but can persist in phases of disease remission. Compared with patients admitted to hospital for non-IBD problems, patients with IBD were more than 5 times more likely to be malnourished, and this was greater in penetrating CD and patients who had undergone bowel resection (Nguyen et al., 2008). The prevalence of malnutrition in IBD depends on the method used to assess it, which includes body mass index (BMI), weight changes, anthropometric measurements and biochemical markers, and the cut-
offs used. One study reported 25%-70% of patients with IBD were malnourished, depending upon the method used (Mijac et al., 2010).

There may be aberrant body composition and impaired muscle strength in IBD despite normal BMI, suggesting the need to assess body composition within malnutrition assessment in IBD (Valentini et al., 2008, Bryant et al., 2013). Malnutrition in IBD is multi-factorial and contributory factors include poor dietary intake resulting from GI symptoms and reduced appetite, increased nutrient requirements due to active inflammation, reduced intestinal nutrient absorption relating to GI inflammation and resections and increased intestinal nutrient losses (Figure 1.2) (Gerasimidis et al., 2011).

Despite the increased risk of malnutrition among patients with IBD, the prevalence of overweight and obesity among IBD is increasing in parallel to the general population. A prospective case-control study revealed 40% of patients with CD had a BMI of \( \geq 25 \text{ kg/m}^2 \), which was not significantly different from healthy controls. High BMI in patients with Crohn’s disease was significantly associated with markers of stable disease (a low CDAI), older age and reduced physical activity, but not with the need for corticosteroids (Nic Suibhne et al., 2013). A retrospective case note review revealed a shorter time to first surgery for CD complications in
patients with a BMI ≥25 kg/m² compared to patients with a BMI ≤18 kg/m² (Hass et al., 2006). In contrast, other studies have observed no adverse effects of obesity on post-surgical outcomes (Guardado et al., 2016) or on healthcare utilisation and IBD-related surgeries (Seminario et al., 2015).

The term malnutrition encompasses micronutrient deficiencies as well as protein-energy malnutrition. The most common micronutrient deficiencies in IBD are iron, vitamin D and vitamin B₁₂. A meta-analysis revealed an overall prevalence of iron deficiency anaemia of 27% in CD and 21% in UC, although a recent large prospective study revealed prevalence of 49% in CD and 20% in UC at diagnosis with the strongest predictor being a high CRP (Hoivik et al., 2014). A further large study supports the high prevalence of anaemia at diagnosis, especially in CD, and showed an association with colonic disease involvement and disease extent in UC (Sjoberg et al., 2014). Vitamin B₁₂ deficiency in IBD likely relates to ileal resections or inflammation, small intestinal bacterial overgrowth and increased requirements. A systematic review revealed that only ileal resections >20 cm predispose to B₁₂ deficiency, whereas CD and UC alone do not (Battat et al., 2014). However, the included studies used only serum B₁₂, of which only a small proportion is biologically active, to detect deficiency. Subsequently, a study utilising holotranscobalamin combined with methylmalonic acid to define B₁₂ deficiency revealed deficiency in 33% in CD and 16% in UC (Ward et al., 2015).

A further measure of malnutrition relevant to IBD is bone health. Patients with IBD are at greater risk of fractures, particularly hip fracture, compared to the general population (Targownik et al., 2013, van Staa et al., 2003). This may relate to inadequate dietary calcium intake or intestinal absorption, reduced BMI and the use of corticosteroids. Inadequate dietary calcium intake may relate to dairy food restrictions, which as discussed in section 1.3.3 are commonly imposed by patients with IBD. In line with this, a cross-sectional study revealed 65% of patients with IBD had inadequate calcium intakes (Vidarsdottir et al., 2016). Due to the greater risk of osteoporosis and fracture, national guidelines suggest that patients with IBD consume 1000 mg/d of calcium, with supplementary calcium administered in patients unable to reach this through dietary sources. Furthermore, vitamin D should be supplemented in patients exhibiting inadequate serum concentrations (Scott et al., 2000).

The interpretation of blood micronutrient concentrations is often troublesome since several are altered during active inflammation as a result of the systemic inflammatory response, during which micronutrients are redistributed and sequestered in tissues. For example, plasma zinc and selenium are significantly associated with CRP and albumin (Ghashut et al., 2016). This obstacle can be overcome by measuring micronutrients in erythrocytes or platelets, although routine access to these analyses may be limited (Oakes et al., 2008).
Numerous studies have investigated nutrient intakes in patients with IBD. These reveal inadequate intakes of a range of nutrients that vary across studies but which include energy, fibre, vitamins C, D, B₁, and B₆, calcium, β-carotene, phosphorous and magnesium, among others (Hartman et al., 2016, Geerling et al., 2000, Filippi et al., 2006, Benjamin et al., 2008, Sousa Guerreiro et al., 2007, Aghdassi et al., 2007, Vagianos et al., 2007). Studies investigating nutrient intakes in IBD vary in terms of dietary assessment, using food frequency questionnaires, diet histories and food diaries. The patients recruited also varies (CD, UC, active, inactive), as does the software used to analyse nutrient intakes. Those studies that have used prospective food records, which minimise recall bias and permit detailed assessment of portion sizes, mainly collect 3-4 day food records. While this is justified in terms of participant burden and investigator burden, a longer diary may better capture dietary fluctuations.

Fructan intakes have been investigated using a food-frequency questionnaire in patients with CD (Anderson et al., 2015). Patients with active CD had significantly lower fructan intakes than patients with inactive CD and healthy controls. However, since this study lacked an assessment of overall energy intake, it remains to be established whether the low fructan intakes in active CD reflect a specific avoidance of high fructan foods (presumably due to GI symptoms induced by these foods) or are simply a result of reduced overall energy intake. This suggests that patients with IBD may already have compromised dietary prebiotic (especially fructan) intake and further restriction with the low FODMAP diet will further reduce this. This study also suggests that patients with IBD may already identify that high FODMAP foods (or high fructan foods) exacerbate GI symptoms and may therefore restrict them, at least during active IBD. However, this requires further investigation.

1.2.3 Patients’ perceptions of diet in IBD

Most patients with IBD consider at least some aspects of diet and nutrition to be important in the disease. Dietary issues experienced by patients encompass the ability to maintain an ideal body weight, dietary GI symptom triggers, lethargy and fatigue, social activities involving food and micronutrient deficiencies (Prince et al., 2011). Some issues, for example maintaining body weight, are particularly experienced by patients with CD and those who have had prior GI surgery. One prospective study of 400 patients with IBD revealed that 48% believed that diet could be the initiating factor in IBD, 57% believed dietary factors could trigger a flare and 60% reported symptom exacerbation following certain foods (Limdi et al., 2016). Specific foods perceived to be inducers of GI symptoms or IBD relapse vary considerably among patients but may include dairy foods, spicy foods, fatty foods, certain fruits and vegetables, wheat, alcohol and high fibre foods (Prince et al., 2011, Limdi et al., 2016, Triggs et al., 2010, Zallot et al., 2013, Kinsey and Burden, 2016). Beliefs regarding the role of diet in IBD may be influenced by IBD
subtype, ethnicity and prior dietary advice and result in dietary restrictions in the majority of patients (Limdi et al., 2016, Jowett et al., 2004b). Food restrictions are also more common in patients with prior GI surgery and stricturing CD (Holt et al., 2017).

Despite most patients considering diet an important factor in IBD, there appears to be a discrepancy between patients’ and their treating physicians’ attitudes and perceptions towards diet. A recent questionnaire survey of 928 Australian patients and IBD specialists revealed 61% of patients felt their IBD specialist disregarded the role of diet and only 26% had received dietary advice from them, meanwhile 98% of gastroenterologists reported providing dietary advice (Holt et al., 2017). Furthermore, in a large survey of UK and New Zealand gastroenterologists significantly more considered dietary exclusion to be effective in the management of IBS than in IBD (Inns and Emmanuel, 2013), which is discordant with the vast majority of IBD patients implementing dietary exclusions.

1.3 Functional gastrointestinal symptoms in IBD

Inflammatory bowel disease typically follows a relapsing and remitting pattern. Periods of remission, as well as the severity and length of relapses, can vary considerably among patients. However, patients in remission are not necessarily asymptomatic. Often, despite objective evidence of inactive disease (e.g. biomarkers of inflammation or endoscopy), patients continue to experience potentially significant GI symptoms, such as abdominal pain, bloating, flatulence, faecal urgency and altered bowel habits. In the absence of GI inflammation and with no identifiable organic cause, these symptoms are referred to as functional GI symptoms (FGS). Persistent FGS can impact psychological well-being and impair quality of life to an equal or greater extent than short-lived IBD relapses. Furthermore, FGS present a treatment dilemma for clinicians since treatment with anti-inflammatory or immune-modulatory medication is inappropriate in this instance. The aetiology of FGS is likely more complex than that of inflammatory symptoms and therefore management of FGS is likely more challenging.

1.3.1 Functional bowel disorders

Bowel disorders, formerly known as ‘functional bowel disorders’ are defined by the Rome IV criteria within the functional gastrointestinal disorders (FGID) (Drossman, 2016). The most common bowel disorder is irritable bowel syndrome (IBS), but functional abdominal bloating, functional diarrhoea and functional constipation also fall under this umbrella (Table 1.3) (Mearin et al., 2016).
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Table 1.3 Rome IV criteria; Bowel disorders, as defined within the functional gastrointestinal disorders

<table>
<thead>
<tr>
<th>C. Bowel disorders</th>
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<tbody>
<tr>
<td>C1. Irritable bowel syndrome</td>
</tr>
<tr>
<td>C2. Functional constipation</td>
</tr>
<tr>
<td>C3. Functional diarrhoea</td>
</tr>
<tr>
<td>C4. Functional abdominal bloating/distension</td>
</tr>
<tr>
<td>C5. Unspecified functional bowel disorders</td>
</tr>
<tr>
<td>C6. Opioid-induced constipation</td>
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</tbody>
</table>

The bowel disorders are chronic GI disorders characterised by GI symptoms occurring in the absence of obvious anatomic or physiologic abnormalities (Mearin et al., 2016). In the movement from the Rome III to the Rome IV criteria, several important changes were made to the IBS criteria, as depicted in Table 1.4. The term 'discomfort' was felt to be variably interpreted and to encompass a different sensation to abdominal pain, and was therefore removed from the definition of IBS. The frequency of abdominal pain has increased and this now must be related to defaecation rather than specifically 'improving' with defaecation, since many patients report a deterioration or no change in GI symptoms following defaecation (Schmulson and Drossman, 2017).

Table 1.4 Rome III vs Rome IV diagnostic criteria for IBS

<table>
<thead>
<tr>
<th>Rome III diagnostic criterion*</th>
<th>Rome IV diagnostic criterion*</th>
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<tr>
<td>Recurrent abdominal pain or discomfort**, on average, at least 3 days per month in the last 3 months, associated with 2 or more of the following criteria:</td>
<td>Recurrent abdominal pain, on average, one day per week in the last 3 months, associated with 2 or more of the following criteria:</td>
</tr>
<tr>
<td>1. Improved with defaecation</td>
<td>1. Related to defaecation</td>
</tr>
<tr>
<td>2. Onset associated with a change in frequency of stool</td>
<td>2. Associated with a change in frequency of stool</td>
</tr>
<tr>
<td>3. Onset associated with a change in form (appearance) of stool</td>
<td>3. Associated with a change in form (appearance) of stool</td>
</tr>
</tbody>
</table>

*Criterion fulfilled for the last 3 months with symptom onset at least 6 months prior to diagnosis

**“Discomfort” means an uncomfortable sensation not described as pain

In pathophysiology research and clinical trials, a pain/discomfort frequency of at least 2 days per week during screening evaluation is recommended for subject eligibility

Irritable bowel syndrome is experienced by around 10% of the UK population, while global prevalence is 11.2% (Lovell and Ford, 2012). More women are affected than men, and younger people are more likely to be affected than those over 50 years old. Poor quality of life at baseline was also associated with the development of IBS within 10 years in 3,659 healthy individuals.
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(Ford et al., 2008). Irritable bowel syndrome is classified into 4 subtypes according to the predominant stool form; diarrhoea-predominant (IBS-D), constipation-predominant (IBS-C), mixed type (IBS-M) and unsubtyped (IBS-U) (Mearin et al., 2016). The pathophysiology of IBS is complex and likely multifactorial, involving psychosocial factors such as stress and anxiety, disordered motility, visceral hypersensitivity, immune dysregulation, GI microbiota aberrations and GI barrier dysfunction (Drossman, 2016). The brain-gut axis describes the interactions between the cognitive centres of the brain via neurotransmitters with the enteric nervous system and vice versa (Jones et al., 2006). This is thought to play a major role in IBS pathophysiology, and in recent years a possible microbiome-gut-brain axis has emerged (illustrated in Figure 1.3 from evidence that the GI microbiota produce neurotransmitters capable of communicating with the central nervous system and that host-derived neurotransmitters influence the GI microbiota (Mayer et al., 2014).

Figure 1.3 Proposed mechanisms of a microbiome-gut-brain axis in IBS pathophysiology (Mayer et al., 2014)

Treatment of IBS begins with explanation and reassurance as to the benign nature of the condition. Pharmacological treatment of IBS is mainly symptom-targeted, including anti-spasmodic agents for abdominal pain, anti-motility agents for diarrhoea and laxatives for constipation. Centrally-acting tricyclic antidepressants and selective serotonin reuptake inhibitors may be effective in reducing abdominal pain (Ford et al., 2014a). Lifestyle modifications, including exercise, stress reduction and improving sleep may be effective,
although objective evidence for these approaches is limited. Based upon evidence of altered GI microbiota in IBS (Rajilic-Stojanovic et al., 2015), commonly characterised by reduced Bifidobacteria, many studies have investigated the potential therapeutic benefit of manipulating the microbiota for GI symptom management. The most recent meta-analysis of probiotics in IBS showed an improvement in GI symptoms with all probiotics, although analysis of individual genera did not reveal any significant benefit of Lactobacillus, Saccharomyces or Bifidobacterium (Ford et al., 2014b). The same meta-analysis concluded that there is currently no evidence for a benefit of prebiotics in IBS.

First-line dietary management of IBS includes, where relevant, general healthy eating, reduction of caffeine, alcohol, fatty foods and spicy foods, and dietary fibre manipulation (McKenzie et al., 2016). However, in the last decade, dietary management of IBS has focussed on dietary fermentable carbohydrate restriction, discussed in detail in section 1.4.

1.3.2 Prevalence of FGS in IBD

Gastrointestinal symptoms are common in patients with inactive IBD, although it is debateable whether patients with IBD can truly meet the criteria for a functional bowel disorder, since the Rome IV guidelines state that functional symptoms occur in the absence of obvious anatomic or physiologic abnormalities (Mearin et al., 2016). However, it could be argued that where GI inflammation is controlled in IBD, persisting GI symptoms could be IBS-like symptoms, which will be referred to as functional gastrointestinal symptoms (FGS) in this thesis.

A systematic review and meta-analysis identified 13 studies reporting FGS prevalence ranging from 20-65%, with a pooled IBS prevalence of 35% in patients in remission (Halpin and Ford, 2012). Further studies of the prevalence of FGS in IBD have since been published (Vivinus-Nébot et al., 2014, Jonefjäll et al., 2016, Berrill et al., 2013, Gracie et al., 2017, Jelsness-Jorgensen et al., 2014, Tomita et al., 2016, Fukuba et al., 2014). Despite abundant data on the prevalence of FGS in IBD (Table 1.5), these studies are heterogeneous in terms of study design (cross-sectional, case-control, prospective cohort), sample size, patients recruited (CD, UC, active or inactive) and criteria used to define IBS or FGS (Manning, Rome, or other). There is also bias associated with varying criteria for disease activity, with some requiring as little as stable medications to define remission and some requiring more reliable endoscopic remission. Furthermore, some studies used clinical disease activity indices to determine activity, which could be raised due to FGS. Therefore, patients with a high score despite being in remission would be categorised as having active IBD and the proportion meeting the criteria for IBS in remission may in fact be greater than 35%. The variety of criteria to define IBS also introduces bias. Interestingly, a study of patients with longstanding UC used a combination of measures to define remission (clinical, endoscopic and faecal calprotectin) and revealed no difference in GI symptoms compared to
controls (Lundgren et al., 2016). However, GI symptoms were measured using the Gastrointestinal Symptom Rating Scale and not the Rome criteria for functional bowel disorders.

One recent study categorised patients according to clinical disease activity indices, IBS criteria and faecal calprotectin. Among the patients with CD, 27.7% met IBS criteria and had a low faecal calprotectin, while the corresponding figure for UC was 19.8% (Gracie et al., 2017). The faecal calprotectin cut-off was 250 μg/g. While this is a commonly cited cut-off for determining active IBD (Lin et al., 2014), it is possible that some patients had low-grade inflammation responsible for the FGS. The prevalence of FGS appears to be greater in CD (35.1%) than in UC (23.9%), which may relate to greater difficulty in identifying active small intestinal inflammation and the lasting effects of complications such as strictures, previous GI surgery and bile acid malabsorption (Halpin and Ford, 2012). Prevalence of FGS does not appear to vary according to initial disease extent or severity in UC (Jonefjall et al., 2015), or disease duration in CD or UC (Berrill et al., 2013).
Table 1.5 Studies reporting the prevalence of functional gastrointestinal symptoms in IBD

<table>
<thead>
<tr>
<th>Author/year</th>
<th>Study design</th>
<th>Population studied</th>
<th>Disease activity of participants</th>
<th>IBD activity assessment</th>
<th>IBS assessment criteria</th>
<th>Prevalence of IBS in remission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isgar et al., 1983</td>
<td>Case-control</td>
<td>UC (n=98)</td>
<td>Remission</td>
<td>Colonoscopy</td>
<td>Manning</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Simrén et al., 2002</td>
<td>Cross-sectional</td>
<td>UC (n=43)</td>
<td>Remission</td>
<td>Colonoscopy; CRP/ESR; Clinical signs</td>
<td>GSRS</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD (n=40)</td>
<td></td>
<td></td>
<td></td>
<td>57</td>
</tr>
<tr>
<td>Minderhoud et al., 2004</td>
<td>Case-control</td>
<td>UC (n=73)</td>
<td>Remission</td>
<td>CAI UC</td>
<td>Manning/Rome II</td>
<td>31.5(^b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD (n=34)</td>
<td></td>
<td>CDAI</td>
<td></td>
<td>41.7(^b)</td>
</tr>
<tr>
<td>Barratt et al., 2005</td>
<td>Cross-sectional</td>
<td>UC (n=88)</td>
<td>Active and remission</td>
<td>Birmingham UC index</td>
<td>Manning/SMDS</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD (n=76)</td>
<td></td>
<td></td>
<td></td>
<td>43.1</td>
</tr>
<tr>
<td>Farrokhyar et al., 2006</td>
<td>Cross-sectional</td>
<td>UC (n=44)</td>
<td>Remission</td>
<td>Modified DAI</td>
<td>Rome II</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD (n=105)</td>
<td></td>
<td></td>
<td></td>
<td>52.4</td>
</tr>
<tr>
<td>Mikocka-Walus et al., 2008</td>
<td>Cross-sectional</td>
<td>UC (n=30)</td>
<td>Active and remission</td>
<td>SCCAI</td>
<td>Rome III</td>
<td>31(^a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD (n=31)</td>
<td></td>
<td>CDAI</td>
<td></td>
<td>31(^a)</td>
</tr>
<tr>
<td>Ansari et al., 2008</td>
<td>Case-control</td>
<td>UC (n=95)</td>
<td>Active and remission</td>
<td>Mayo score</td>
<td>Rome II</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Piche et al., 2010</td>
<td>Cross-sectional</td>
<td>CD (n=92)</td>
<td>Remission</td>
<td>CDAI; colonoscopy: CRP/ESR</td>
<td>Rome III</td>
<td>45.6</td>
</tr>
<tr>
<td>Keohane et al., 2010</td>
<td>Cross-sectional</td>
<td>UC (n=44)</td>
<td>Remission</td>
<td>PGA; CRP; No steroids or biologics in 6/12; CDAI/UCAI</td>
<td>Rome II</td>
<td>38.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD (n=62)</td>
<td></td>
<td></td>
<td></td>
<td>59.7</td>
</tr>
<tr>
<td></td>
<td>Case-control</td>
<td>UC (n=63)</td>
<td>Active and remission</td>
<td>PGA</td>
<td>Rome III</td>
<td>20.4(^a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD (n=93)</td>
<td></td>
<td></td>
<td></td>
<td>20.4(^a)</td>
</tr>
</tbody>
</table>

\(^a\) Significant difference with healthy controls.
\(^b\) Significant difference with healthy controls.
\(^c\) Significant difference with disease controls.

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<table>
<thead>
<tr>
<th>Author/year</th>
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<th>IBS assessment criteria</th>
<th>Prevalence of IBS in remission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vivinus-Nébot et al., 2014</td>
<td>Cross-sectional</td>
<td>UC (n=18) CD (n=31)</td>
<td>Remission</td>
<td>PGA; CRP/ESR; No steroids 1 year; CDAI/UCAI; Colonoscopy</td>
<td>Rome III</td>
<td>38</td>
</tr>
<tr>
<td>Jonefjäll et al., 2013</td>
<td>Prospective</td>
<td>UC (n=94)</td>
<td>Remission</td>
<td>Mayo score</td>
<td>Rome II</td>
<td>27</td>
</tr>
<tr>
<td>Berrill et al., 2013</td>
<td>Cross-sectional</td>
<td>UC (n=101) CD (n=68)</td>
<td>Active and remission</td>
<td>SCCAI and CRP</td>
<td>Rome III</td>
<td>58c</td>
</tr>
<tr>
<td>Fukuba et al., 2014</td>
<td>Cross-sectional</td>
<td>UC (n=172) CD (n=68)</td>
<td>Remission</td>
<td>CAI</td>
<td>Rome III</td>
<td>27</td>
</tr>
<tr>
<td>Gracie et al., 2017</td>
<td>Cross-sectional</td>
<td>UC (n=172) CD (n=206)</td>
<td>Remission</td>
<td>HBI/SCCAI; FCP</td>
<td>Rome III</td>
<td>20</td>
</tr>
<tr>
<td>Henrikson et al., 2018</td>
<td>Prospective</td>
<td>UC (n=260)</td>
<td>Active and remission</td>
<td>Mayo score; FCP</td>
<td>Rome III</td>
<td>25</td>
</tr>
<tr>
<td>Tomita et al., 2016</td>
<td>Case-control</td>
<td>UC (n=40) CD (n=107)</td>
<td>Remission</td>
<td>CAI/CDAI</td>
<td>Rome III</td>
<td>17.5</td>
</tr>
<tr>
<td>Jelsness-Jorgensen et al., 2014</td>
<td>Cross-sectional</td>
<td>UC (n=61) CD (n=28)</td>
<td>Remission</td>
<td>SCCAI/SCDAI</td>
<td>Rome II/Rome III</td>
<td>22e</td>
</tr>
</tbody>
</table>

a Subgroup analyses for CD and UC not reported, b According to Rome II criteria, c Patients in remission only, d Continuous GSRS score compared between groups, e Rome III criteria

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; GSRS, Gastrointestinal Symptom Rating Scale; CDAI, Crohn’s Disease Activity Index; SCDAI, short Crohn’s Disease Activity Index; CAI, Clinical Activity Index; HBI, Harvey-Bradshaw Index; DAI, Disease Activity Index; SCCAI, Simple Clinical Colitis Activity Index; PGA, Physician Global Assessment; UCAI, Ulcerative Colitis Activity Index; FCP, faecal calprotectin
1.3.3 Pathogenesis of FGS in IBD

The pathogenesis of FGS in IBD and the explanation for a greater prevalence compared to the general population (35% vs. 10.0%) remains elusive. Statistically, due to the high prevalence of IBS in the general population, some patients with IBD will also have IBS. In support of this, females and those with anxiety are more likely to have IBS in IBD, mirroring the general population (Berrill et al., 2013, Jonefjall et al., 2013). Furthermore, physiological features observed in IBS have also been detected in patients with inactive IBD with FGS. For example, rectal perception with electronic barostat was shown to correlate positively with FGS in patients with inactive UC (van Hoboken et al., 2011), similar to patients with IBS (Chang et al., 2000). However, co-existing IBS seems unlikely to be the sole explanation, given the increased prevalence of IBS in IBD compared to the general population.

An alternative hypothesis is that FGS in IBD may represent low-grade inflammation. One study of 62 patients with inactive IBD revealed that faecal calprotectin concentrations were elevated in patients fulfilling the Rome II criteria for IBS (Keohane et al., 2010), while several subsequent studies observed no difference in faecal calprotectin between patients with and without IBS-like symptoms (Berrill et al., 2013, Jonefjall et al., 2013, Jonefjäll et al., 2016, Lundgren et al., 2016). In line with the latter studies, a similar prevalence of IBS-like symptoms has been observed in patients with no signs of inflammation on colonic biopsies (i.e. patients in deep remission) (29%) compared to the whole cohort (27%) (Henriksen et al., 2017), although a prior study revealed a lower rate of IBS-like symptoms when more stringent histological remission thresholds were applied (Fukuba et al., 2014). Despite no difference in faecal calprotectin, one study revealed elevated serum cytokines including IL-6, IL-8, IL-10 and IL-13 in patients with FGS compared to those without (Jonefjäll et al., 2016). Together, these findings suggest that low-grade inflammation may be responsible for IBS-like symptoms in some, but not all, patients with IBD.

Numerous studies associate IBS-like symptoms in IBD with heightened anxiety and depression and poorer HR-QOL, although the direction of this association is unclear (Jonefjäll et al., 2016, Simrén et al., 2002, Jonefjall et al., 2015, Bryant et al., 2011). It is accepted that the brain-gut axis functions in a bidirectional manner and it is possible that, similar to IBS in the general population, psychological comorbidity may play a role in inducing FGS in IBD, either directly or through the GI microbiota (Jones et al., 2006). The burden of living with a chronic condition such as IBD is known to impact psychological well-being (Walker et al., 2008), therefore it is possible that psychological comorbidity induces FGS in some patients in remission.

Surprisingly, to date there has been no investigation of the GI microbiota in patients with FGS in IBD, but given the apparent dysbiosis in both IBD and IBS, it seems logical to investigate whether a distinct microbial profile exists in patients with inactive IBD and FGS.
It has been proposed that, resulting from the effects of underlying IBD, FGS in IBD have a different pathophysiology from IBS in the general population. This is supported by evidence of pro-inflammatory features such as an enhanced TNF-α response to bacterial lipopolysaccharide and elevated cytokines in at least a subset of patients with IBS in IBD (Vivinus-Nébot et al., 2014, Jonefjäll et al., 2016). It could be that the inflammatory response during active IBD upregulates components of the brain-gut axis, resulting in disordered gut motility, visceral hypersensitivity and dysregulated brain-gut signalling (Quigley, 2016). Transient receptor potential vanilloid type 1 (TRPV1) is expressed on primary afferent neurones and is activated by capsaicin, lipids, resinferatoxin, endocannabinoids and noxious heat (Caterina et al., 1997). This leads to pain sensation and initiation of neurogenic inflammation and is thought to contribute to visceral hypersensitivity. Expression of TRPV1 in colonic mucosa is greater in patients with IBD experiencing pain and in patients with IBS, where TRPV1 expression correlated with abdominal pain severity (Akbar et al., 2008, Yiangou et al., 2001). In patients with histologically inactive IBD, TRPV1 expression was 5.3-fold higher in patients with abdominal pain compared to without abdominal pain (Akbar et al., 2010). The potential role of TRPV1 in visceral hypersensitivity following active inflammation is supported by a murine study in which pain-related behaviours following experimental colitis correlate with TRPV1 expression, and is reduced following TRPV1 deletion (Lapointe et al., 2015). Furthermore, upregulated transient receptor potential ankyrin-1 may mediate visceral pain following experimental colitis (Yang et al., 2008). The role of these molecules in the pathophysiology of IBS-like symptoms in patients following resolution of active inflammation remains to be explored.

1.3.4 Treatment of FGS in IBD

Effective management of IBS in IBD is important given the likely impact of these symptoms on HR-QOL. Furthermore, persistent GI symptoms are likely to lead to a greater number of physician visits, thus increasing the healthcare resource burden associated with IBD, although objective evidence for this is lacking.

Theoretically, in the absence of active inflammation, management of IBS in IBD could encompass strategies used in IBS in the general population, which in terms of pharmacological and first-line dietary management has been discussed in section 1.3.1 (McKenzie et al., 2016).

Few studies to date have investigated the management of FGS in IBD specifically. A randomised controlled trial revealed that a mindfulness and cognitive behavioural therapy intervention directly improved HR-QOL in patients with inactive IBD and IBS (Berrill et al., 2014). As will be discussed in section 1.4.6, dietary fermentable carbohydrate restriction is the most promising management strategy for IBS in IBD.
1.4 The low FODMAP diet

1.4.1 Introduction

The manipulation of dietary carbohydrates has been used to manage FGS for several years. For example, a meta-analysis revealed 22 RCTs investigating dietary fibre manipulation for the management of IBS symptoms, demonstrating that soluble fibre may be of benefit for global GI symptoms compared with placebo (Nagarajan et al., 2015).

Studies suggesting fructose, lactose and sorbitol to be GI symptom inducers in functional bowel disorders were first published almost 30 years ago (Rumessen and Gudmand-Hoyer, 1988, Fernández-Bañares et al., 1993). Fructans were also identified as capable of inducing GI symptoms. A retrospective study revealed improvements in GI symptoms following dietary fructose and fructan restriction in patients with fructose malabsorption, albeit with obvious limitations in study design (Shepherd and Gibson, 2006). However, there is a substantial placebo response in IBS and foods contain a complex a mixture of nutrients and bioactive components, any of which could affect GI symptoms. Therefore, it remained unclear whether fermentable carbohydrates were responsible for GI symptoms. To address this, an Australian group conducted a randomised, placebo-controlled, crossover re-challenge trial to investigate whether pure fermentable carbohydrate challenges induced GI symptoms on the background of a low FODMAP diet. Fructans and fructose both induced GI symptoms compared to a glucose placebo, and greater symptom severity was evident following consumption of a mixture of fructans and fructose, suggesting an additive effect of different FODMAPs (Shepherd et al., 2008). From here, the concept of a broad dietary restriction of poorly-absorbed, osmotically active and fermentable short-chain carbohydrates was proposed, involving the restriction of oligosaccharides (fructans and the rapidly fermented galacto-oligosaccharides; GOS), disaccharides (lactose), monosaccharides (fructose) and polyols (sugar alcohols including sorbitol and mannitol). Thus, this approach has been termed the ‘low FODMAP diet’ (Staudacher and Whelan, 2017).

<table>
<thead>
<tr>
<th>Fermentable carbohydrate</th>
<th>Major food sources (UK diet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructans</td>
<td>Bread, pasta, wheat-based breakfast cereals, onions, garlic</td>
</tr>
<tr>
<td>GOS</td>
<td>Pulses, cashew nuts, pistachio nuts</td>
</tr>
<tr>
<td>Lactose</td>
<td>Milk, yoghurt</td>
</tr>
<tr>
<td>Fructose</td>
<td>Certain fruits e.g. mango, honey, certain sweetened products</td>
</tr>
<tr>
<td>Polyols</td>
<td>Stone fruits, mushrooms, cauliflower, sugar-free chewing gum, diabetic products</td>
</tr>
</tbody>
</table>

GOS, galacto-oligosaccharides
Table 1.6 presents an overview of the dietary sources of each fermentable carbohydrate in a UK diet. The remainder of section 1.3 will describe the individual carbohydrates restricted on the low FODMAP diet, the proposed mechanisms of action of the diet, the evidence for the effectiveness of the diet in both IBS and IBD and the potential safety considerations of the diet with reference specifically to IBD.

1.4.2 Fermentable carbohydrates
Dietary carbohydrates include those that are rapidly digested and/or absorbed in the small intestine (glucose, sucrose and starch), as well as those that are partially or totally undigested in the small intestine due to a limited expression or total lack of the appropriate enzymes to hydrolyse them to monosaccharides for absorption. Carbohydrates that are minimally digested and absorbed include non-starch polysaccharides (NSP), resistant starch (RS), certain oligosaccharides (such as fructans and GOS), polyols, and in some individuals, fructose and lactose (Cummings and Stephen, 2007). Under normal physiologic conditions, little rapidly digested carbohydrate (e.g. glucose, sucrose, starch) enters the colon, whereas most of the minimally digested and absorbed carbohydrates (e.g. NSP, RS, oligosaccharides) reach the colon intact and are utilised to varying degrees as substrates for bacterial fermentation. The carbohydrates restricted during a low FODMAP diet fall under the umbrella of minimally or incompletely digested carbohydrates. Each of the short-chain carbohydrates restricted on a low FODMAP diet are reviewed below.

1.4.2.1 Fructans
Fructans include inulin and oligofructose and are polymers of fructose units linked by β2→1 bonds, usually with a terminal glucose unit. The term 'inulin' refers to fructans with a degree of polymerisation (DP) 2-60, with an average DP of 12, while oligofructose is typically DP 2-8, with an average DP 3-6 (Roberfroid et al., 2010). Fructans are produced within certain plants through the action of fructosyltransferases and act as a carbohydrate storage reservoir by the plant (Vijn and Smeekens, 1999). Humans lack the appropriate fructanases to hydrolyse the fructosyl-fructose links and thus, it has been demonstrated through patients with an ileostomy that >85% of fructans pass through the small intestine intact and enter the colon (Barrett et al., 2010, Knudsen and Hessov, 1995, Ellegard et al., 1997).

Some foods, such as artichokes and chicory, are particularly rich sources of fructans. However, the majority of fructans in a Western diet derive from wheat, onions and garlic, since these foods are consumed in large quantities (Table 1.6) (van Loo et al., 1995, Dunn et al., 2011, Moshfegh et al., 1999, Anderson et al., 2015). The fructan content of foods may vary depending on variety,
soil characteristics in the place of cultivation, and processing applied. Typical daily intake of fructans in the UK is around 4.0 g/day in healthy individuals (Dunn et al., 2011, Anderson et al., 2015), 2.3-5.0 g/day in patients with IBS (Staudacher et al., 2012, Bohn et al., 2015, Staudacher et al., 2017b) and 2.9-3.6 g/day in patients with CD (depending on disease activity) (Anderson et al., 2015). It should be noted that studies assessing the intake of fermentable carbohydrates in IBS are scarce and mainly utilise food frequency questionnaires rather than a more robust prospective food record.

In the colon, fructans are fermented by bacteria, with inulin and oligofructose displaying slightly different fermentation profiles for gas and SCFA production (Koecher et al., 2014). Fructans are considered ‘prebiotic’, defined as ‘a substrate that is selectively utilised by host microorganisms conferring a health benefit’ (Gibson et al., 2017). Bifidobacteria and Lactobacilli are the most widely regarded as exerting health benefits and their growth is stimulated by fructans. It has been suggested from in vitro studies that inulin has a more pronounced and prolonged Bifidogenic effect than oligofructose (van de Wiele et al., 2007, Pompei et al., 2008). Bacterial fermentation of fructans also results in SCFA generation, which have beneficial effects on the host, and the stimulation of bacterial growth increases biomass which in turn contributes to stool bulking (Smith et al., 2013). Furthermore, increasing evidence from murine and human studies suggests that fructans can increase mineral absorption in the GI tract (for example, calcium) and have lipid-lowering effects (Roberfroid et al., 2010, Van Loo et al., 1999).

1.4.2.2 Galacto-oligosaccharides

Non-digestible oligo-saccharides also include GOS, polymers of galactose units linked by varying bonds. Galacto-oligosaccharides derived from plants consist of galactose units linked by α1→6 bonds, with a terminal sucrose (α-GOS) (Cummings and Stephen, 2007). The major types of α-GOS are raffinose, stachyose and verbascose and are found predominantly in pulses and nuts (Table 1.6). The human GI tract does not possess α-galactosidase to hydrolyse α-GOS and therefore >85% enters the colon intact (Saunders and Wiggins, 1981). Typical intakes of α-GOS are 1.0 g/day in patients with IBS in the UK and around 0.5 g/day in patients with IBS in Sweden (Staudacher et al., 2012, Bohn et al., 2015).

A distinction should be made between the plant-derived α-GOS and β-GOS or trans-galacto-oligosaccharides, produced commercially through the action of β-galactosidase on lactose (Wilson and Whelan, 2017). In contrast to α-GOS, which, due to a variety of bonds, can be fermented by a range of GI bacteria, β-GOS selectively stimulate the growth of Bifidobacteria and are therefore argued to be prebiotics (Vulevic et al., 2004).
Similar to fructans, GOS exert benefits on health through the stimulation of beneficial GI bacteria and stool bulking (Fernando et al., 2010). Furthermore, systemic benefits of α-GOS have been observed. Bean consumption has been shown to reduce serum total and LDL cholesterol in individuals with raised cholesterol. However, it is difficult to attribute the health benefits of pulses to α-GOS specifically, rather than other constituents such as fibre or polyphenols (Trinidad et al., 2010). Addressing this, supplementation with pure α-GOS, that lacks the other constituents of beans, reduced appetite, food intake and inflammatory markers in obese adults compared to controls (Morel et al., 2015).

1.4.2.3 Lactose

Lactose is a disaccharide of galactose and glucose linked by a β1→4 bond and is found exclusively in mammalian milks. Lactose is hydrolysed by lactase situated in the brush border of the small intestine. Unsurprisingly, the major dietary sources of lactose are dairy products but a small amount is added during manufacturing of certain foods.

Lactose malabsorption has been demonstrated through hydrogen breath testing in 51% of patients with functional bowel disorders (Wilder-Smith et al., 2013). However, malabsorption does not necessarily result in intolerance, and in line with this in the aforementioned study malabsorption did not correlate with symptoms evoked during testing. Similarly, breath hydrogen was comparable between patients with IBS and healthy controls following a lactose challenge, while lactose intolerance (lactose malabsorption plus the presence of symptoms upon ingestion) was more frequent in IBS (Zhu et al., 2013). Together, these findings suggest lactose malabsorption can occur without intolerance, and does not appear to be elevated in patients with functional bowel disorders compared to healthy controls.

A systematic review and meta-analysis has shown greater odds of lactose malabsorption among patients with IBD compared to healthy controls (OR 1.61, P=0.048), with sub-group analysis revealing this to be significant in CD only (Szilagyi et al., 2016). This may relate to the effects of previous small intestinal inflammation on lactase concentration in the brush border. Concordant with studies in IBS, lactose malabsorption and intolerance were often discrepant.

1.4.2.4 Fructose

Fructose is a hexose sugar that is present in increasing quantities in the Western diet. It is absorbed in the small intestine via facilitative transport. The first is via the facultative transporter GLUT5, which appears to be specific to fructose, expressed predominantly on the apical membrane of intestinal epithelial cells and may be up-regulated by dietary fructose
consumption. Fructose absorption via this route is dose dependent and displays inter-individual variation, possibly relating to variable GLUT5 expression (Jones et al., 2011).

The second mechanism of fructose absorption is via the facilitative GLUT2 transporter, which co-transport glucose and fructose across both the apical and basolateral membranes of intestinal epithelial cells and is up-regulated by the presence of glucose in the lumen. Thus, the presence of glucose likely enhances fructose absorption through the recruitment of GLUT2 to the brush border, and fructose in excess of glucose likely relies upon the GLUT5 pathway for absorption (Jones et al., 2011). In support of this, adding glucose to fructose solutions or fruit juices improves fructose absorption (measured using hydrogen breath tests) in healthy controls (Truswell et al., 1988) and patients with functional bowel disorders (Tuck et al., 2017).

Fructose malabsorption is prevalent in healthy individuals, with >50% incompletely absorbing a 25-50 g dose of fructose, although fructose intolerance can occur independently of malabsorption (Gibson et al., 2007).

Fructose derives predominantly from fruits, fruit juices and honey, but is also added during food manufacturing in varying forms including fructose, fructose syrup, glucose-fructose syrup and high-fructose corn syrup. Fructose intakes vary across the world depend upon food processing practices. For example, fructose intake in the US appears much higher than in UK (49 g vs. 12-17 g/day) (Marriott et al., 2009, Staudacher et al., 2012, Staudacher et al., 2017b), likely a result of the use of high-fructose corn syrup in the US. However, these figures refer to total fructose intake, whereas the intake of fructose in excess of glucose (i.e. a greater than 1:1 ratio of fructose:glucose) is likely to be considerably lower.

1.4.2.5 Polyols

Polyols, or sugar alcohols, such as sorbitol, mannitol and xylitol are naturally present in some foods and can also be added during manufacturing. For example, sugar-free chewing gum contains a considerable quantity of xylitol. Polyols are passively absorbed to varying degrees in the small intestine, and absorption is influenced by the molecular size of the polyol, pore size in different regions of the small intestine and the presence of organic disease. An ileostomy study showed that 24-40% of a 30 g dose of each of sorbitol, isomalt and maltitol was recovered in ileostomy effluent, and absorption was dose-dependent (Langkilde et al., 1994). Studies using breath hydrogen have shown that 67-71% of healthy volunteers and 60% of IBS patients incompletely absorbed 10 g sorbitol (Yao et al., 2014, Hyams, 1983).
The major naturally-occurring polyols are sorbitol, found in stone fruits, avocado and broccoli, and mannitol, found in mushrooms and cauliflower (Yao et al., 2014). Owing to poor intestinal absorption, polyols have lower caloric value than sucrose and are therefore used as sweeteners in sugar-free and reduced-calorie products. Furthermore, polyols do not increase the risk of dental caries and are thus added to sugar-free chewing gum at relatively high doses (Zumbe et al., 2001). Polyol intakes in the UK are estimated at around 1 g/d in IBS (Staudacher et al., 2012, Staudacher et al., 2017b, Bohn et al., 2015) and a recent survey based on UK National Diet and Nutrition Survey data suggested a daily intake of 2.7 g polyols in the general population (Tennant, 2014). The discrepancy between the IBS and general population intakes likely reflects differences in data collection, with the former established through individual-level food diaries and food frequency questionnaires and the latter a population-based survey of estimated exposure.

### 1.4.3 Mechanisms of effects of FODMAPs on gastrointestinal symptoms

The mechanisms through which fermentable carbohydrates induce GI symptoms have been increasingly investigated in recent years, following evidence that the diet is effective in managing FGS. Figure 1.4 illustrates the major hypothesised mechanisms through which FODMAPs induce GI symptoms.

#### 1.4.3.1 Small intestinal water

Collectively FODMAPs increase small bowel water content. A study of patients with an ileostomy showed a greater effluent volume and water content following a 4-day high FODMAP diet compared to a 4-day low FODMAP diet, during which participants were provided with all food consumed (Barrett et al., 2010). However, this study did not investigate the effect of individual FODMAPs on small bowel water, only a complete diet.

The effects of the smaller, osmotically active FODMAPs (fructose, lactose and polyols) on small bowel water have been investigated on the basis that slow absorption results in their high luminal concentration, drawing water into the lumen along a concentration gradient. A study in healthy volunteers demonstrated through functional MRI scanning that a 40 g fructose challenge led to greater small bowel water compared to rapidly-absorbed glucose, while inulin had no effect on small bowel water (Murray et al., 2014). These results were mirrored in a more recent similar study, reporting a significant increase in small bowel water following a 40 g fructose challenge in both healthy volunteers and patients with IBS (Major et al., 2017). Interestingly, following the fructose challenge GI symptom induction was observed only in patients with IBS, demonstrating for the first time that an increase in small bowel water per se does not induce symptoms, but rather an increase in small bowel water in the context of visceral hypersensitivity.
In both of the aforementioned studies, the addition of glucose to the fructose challenge significantly reduced small bowel water, further confirming that glucose facilitates fructose absorption, as described above.

Although these studies indicate that inulin has little effect on small bowel water, the effect of short-chain oligofructose on small bowel water is yet to be clarified, which may differ to inulin due to smaller molecular weight. Oligofructose contributes a greater proportion of dietary fructans than inulin (Dunn et al., 2011, Anderson et al., 2015), and therefore the physiological effects of these carbohydrates is clinically relevant. Furthermore, the pure carbohydrate challenges in the described mechanistic studies were administered to participants who were fasted, and were not given alongside a meal. The effects of the carbohydrates may differ when given in pure form compared to when they are consumed as part of a food, where the complex mixture of nutrients and other dietary components may influence osmolality and gastric emptying (Chaudhuri and Fink, 1991).

Figure 1.4 The proposed mechanisms of GI symptom improvement with a low FODMAP diet. Poorly absorbed, fermentable carbohydrates, such as fructans, fructose and lactose are eaten within a normal diet. Smaller, osmotically active carbohydrates (fructose, lactose and polyols) encourage water to enter the lumen (panel 1). Fructans and GOS reach the colon intact where they undergo bacterial fermentation, with the production of hydrogen, methane and carbon dioxide, along with SCFA (panel 2). The gases and water in the lumen cause luminal distension which can be felt as GI symptoms in the context of visceral hypersensitivity (panel 3). GOS, galacto-oligosaccharides; SCFA, short-chain fatty acids. From Staudacher and Whelan (Staudacher and Whelan, 2017)

Similar effects on small bowel water have been demonstrated for the polyol mannitol. Significantly greater small bowel water was observed on MRI in healthy volunteers following 17.5 g mannitol compared to a glucose placebo challenge (Marciani et al., 2010). The effect of sorbitol on small bowel water has not been directly measured using MRI, although increased ileostomy output was demonstrated following the consumption of sorbitol, isomalt and maltitol challenges (Langkilde et al., 1994).


1.4.3.2 **Fermentation and gas production**

The second mechanism through which FODMAPs are hypothesised to induce GI symptoms is bacterial fermentation of undigested carbohydrates reaching the colon, the associated hydrogen, carbon dioxide and methane generation, and the resulting luminal distension. As described above, all FODMAPs have the capacity to reach the colon for bacterial fermentation due to almost non-existent hydrolysis in the small intestine (fructans, GOS), varying expression of enzymes for hydrolysis (lactose) or limited absorption in the small intestine (fructose, polyols).

Hydrogen produced during colonic bacterial fermentation is absorbed into the blood and eventually expelled in the breath and is the major source of breath hydrogen. This can therefore be used as a marker of substrate fermentation by the colonic microbiota, although the reproducibility and clinical relevance of hydrogen breath tests have been questioned in recent years (Yao and Tuck, 2017, Wilder-Smith et al., 2013). Nonetheless, a 2-day high FODMAP diet increased breath hydrogen in healthy controls and patients with IBS compared to a 2-day low FODMAP diet, although GI symptoms were significantly greater in IBS only during the high FODMAP diet (Ong et al., 2010).

An increase in colonic gas following FODMAPs is supported by studies using MRI. Greater colonic gas volume was observed in healthy individuals following 40 g inulin, but not fructose, challenge, suggesting that a greater proportion of certain FODMAPs reach the colon intact and are fermented to a greater extent than others (Murray et al., 2014). These findings were replicated in a further study in healthy volunteers and patients with IBS. In addition, fructose significantly increased colonic gas compared to glucose in patients with IBS. Interestingly, peak symptom intensity correlated with peak colonic gas in patients meeting a pre-defined symptom threshold, indicating for the first time an association between the physiological effects of FODMAPs and GI symptoms (Major et al., 2017).

1.4.3.3 **Other possible mechanisms of effects of FODMAPs on gastrointestinal symptoms**

There are mechanisms, other than a reduction in luminal water and gas, through which the low FODMAP diet may improve GI symptoms, although currently these are simply observations from RCTs of the low FODMAP diet and require further research to establish these mechanisms. One is via changes to the GI microbiota, which are known to occur following a low FODMAP diet in IBS (Staudacher et al., 2017b, Staudacher et al., 2012, Halmos et al., 2014a, McIntosh et al., 2016). Following a 3-week low FODMAP diet in patients with IBS, the abundance of a hydrogen-utilising genus *Adlercreutzia* was significantly higher than compared to a 3-week high FODMAP diet (McIntosh et al., 2016). This may suggest that as well as reduced fermentation of FODMAPs and reduced hydrogen production, the response to the low FODMAP diet may also relate to a
greater abundance of bacteria capable of using hydrogen, thus further reducing hydrogen concentration.

The patients in the low FODMAP and high FODMAP diet groups could be discriminated via metabolomic analysis of urine, on the basis of three metabolites including histamine, which was significantly lower on the low FODMAP diet (McIntosh et al., 2016). Histamine is produced by mast cells, which have previously been shown to be elevated in number, activated and in closer proximity to nerve fibres in IBS mucosa compared to healthy controls (Barbara et al., 2004, Park et al., 2006). Furthermore, correlations were observed between microbiota, GI symptoms, hydrogen production and the urine metabolome, indicating that these factors may have some role in mechanisms of the diet.

1.4.4 The low FODMAP diet delivery

The low FODMAP diet requires significant dietary changes for most patients. Foods are excluded from most of the recognised food groups, including cereals/starchy foods, fruits, vegetables and dairy. Thus far, evidence for the low FODMAP diet has predominantly involved dietitian-led advice (Staudacher et al., 2017b, Staudacher et al., 2012, Halmos et al., 2014b, De Roest et al., 2013, Chumpitazi et al., 2015, Pedersen et al., 2017), although two studies have utilised nurse-led low FODMAP dietary advice (Mazzawi et al., 2013, Ostgaard et al., 2012). Neither of the nurse-led studies were RCTs and thus do not provide high-quality evidence for the effectiveness of this method of low FODMAP diet delivery. Therefore, it is currently unclear whether other methods of diet delivery (other healthcare professionals e.g. nurses and doctors, leaflets or mobile phone applications) would be equally as effective as dietitian-led (O’Keeffe and Lomer, 2017). It is for this reason that IBS guidelines specify that the low FODMAP diet should preferably be delivered by a healthcare professional with expertise in dietary management of IBS (NICE, 2008).

The low FODMAP diet consists of three phases. A FODMAP restriction phase of 4 weeks is recommended (Whelan et al., 2018), although in clinical practice it can last up to twelve weeks depending upon patient preference and availability of appointments. Following a thorough assessment including anthropometry, medical history, diet history and symptom assessment, patients are educated on high-FODMAP foods to exclude and low-FODMAP foods to consume instead. Certain foods (e.g. avocado, broccoli), contain intermediate quantities of FODMAPs and are permitted in limited portion sizes. Patients are usually provided with written resources containing information on restricted and permitted foods and ingredients, recipes and additional information such as high-fibre foods. Patients experiencing significant GI symptom improvement following the restriction phase proceed to the FODMAP reintroduction phase, the aim of which is twofold. Firstly, reintroducing individual high-FODMAP foods in isolation while
continuing to exclude other high-FODMAP foods enables patients to establish tolerance to the reintroduced foods. Secondly, the personalisation phase that follows the reintroduction phase increases the variety of the diet and reduces the risk of nutritional inadequacy. The final phase is personalisation, during which tolerated foods are consumed, while those inducing significant GI symptoms upon reintroduction continue to be excluded, with the hope of managing GI symptoms in the long term.

1.4.5 Evidence for the low FODMAP diet in IBS

At least eight RCTs of the effect of the low FODMAP diet on GI symptoms in IBS have now been published (Table 1.7). There are four meta-analyses of the low FODMAP diet in IBS, indicating the growing number of trials in this field (Marsh et al., 2016, Rao et al., 2015, Varju et al., 2017, Altobelli et al., 2017). Many of the studies are retrospective or prospective uncontrolled trials and many of the RCTs have clear methodological limitations, as outlined by a recent systematic review (Krogsgaard et al., 2017). Several RCTs have been published, including one blinded feeding study and one large placebo-controlled dietary advice trial. The RCTs of the low FODMAP diet in IBS will be discussed below.

The first RCT of the low FODMAP diet in IBS was conducted by our group in the UK and investigated the effect of low FODMAP dietary advice compared to continuing with a habitual diet (Staudacher et al., 2012). More patients in the low FODMAP diet group (68%) reported adequate relief of FGS compared with habitual diet (23%), and more experienced an improvement in bloating, borborygmi, faecal urgency and overall symptoms during the trial, compared to the control group. Although this trial provided the first RCT evidence of the effectiveness of the low FODMAP diet in IBS there were considerable limitations, most importantly that the control group received no treatment and, as such, participants could not be blinded to group allocation. Designing or choosing suitable control dietary advice is extremely challenging, however the lack of treatment in the control group means that a placebo response cannot be accounted for and increases the risk of expectation bias in both groups (Staudacher et al., 2017a). This is particularly problematic with regards to functional bowel disorders, where placebo responses are considerable, and the low FODMAP diet that involves intensive counselling from a dietitian and is likely susceptible to enhanced placebo responses (Elsenbruch and Enck, 2015). Our group recently addressed the issue pertaining to placebo control treatments in low FODMAP diet trials by performing a trial comparing low FODMAP dietary advice to sham dietary advice in 104 patients with IBS. Gastrointestinal symptom severity was significantly lower and significantly more patients were defined as responders to the low FODMAP (73%) compared to the sham diet (42%) (Staudacher et al., 2017b). To date, this is the only trial of the low FODMAP diet in IBS to allow an assessment of the placebo response, since
the sham dietary advice was designed to generate a placebo response through dietary advice, while maintaining unchanged nutrient and FODMAP intakes.

A feeding study compared the low FODMAP diet (3.1 g total FODMAPs) to a diet with a FODMAP content reflective of a 'typical' Australian diet (23.7 g total FODMAPs) (Halmos et al., 2014b). Participants completed both diets for 3 weeks in a random order, during which all food was provided, separated by a 3-week washout period. During the low FODMAP diet arm, overall GI symptoms, and individual symptoms including bloating, pain and flatulence, were significantly lower in severity compared to the typical Australian diet. Such ‘feeding’ trial design enables blinding of participants since foods can be designed to be indistinguishable in appearance and taste on the different diets and also enhances compliance. While this design is ideal for proof-of-concept, it does not mimic the effectiveness of the diet in clinical practice where patients are required to implement dietary advice. It is unlikely that in clinical practice, patients will achieve the low FODMAP intakes observed in this feeding study, in which very low FODMAP foods are provided. This is evident from low FODMAP dietary advice trials (Staudacher et al., 2017a, Staudacher et al., 2017b, Staudacher et al., 2012). Therefore, symptom responses in clinical practice may be lower than those observed in this trial.
### Table 1.7 Randomised controlled trials and randomised comparative trials of the effectiveness of the low FODMAP diet in adults with IBS and IBD

<table>
<thead>
<tr>
<th>Reference</th>
<th>Trial design</th>
<th>Comparator</th>
<th>Diet delivery</th>
<th>Participants</th>
<th>Duration</th>
<th>Symptom assessment</th>
<th>Main findings at end of trial</th>
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<tbody>
<tr>
<td><strong>Unblinded, IBS</strong></td>
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<tr>
<td>Staudacher et al., 2012</td>
<td>Randomised controlled trial</td>
<td>Habitual diet</td>
<td>Dietary advice, dietitian-led</td>
<td>Rome III IBS with bloating or diarrhoea LFD n=19 Habitual diet n=22</td>
<td>4 weeks AR question GSRS BSFS</td>
<td>↑% patients reporting AR in LFD (68%) vs. habitual (23%) ↑% patients reporting improved bloating, borborygmi, urgency and overall symptoms in LFD vs. habitual ↓ Stool frequency and ↑% normal stool consistency in LFD vs. habitual ↓ Relative Bifidobacteria abundance in LFD vs. habitual</td>
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<tr>
<td>Pedersen et al., 2014</td>
<td>Randomised controlled trial</td>
<td>LGG</td>
<td>Habitual diet</td>
<td>Rome III IBS LFD n=42 LGG n=41 Habitual diet n=40</td>
<td>6 weeks IBS-SSS (web-based)</td>
<td>↓ Total IBS-SSS score in low FODMAP diet vs. habitual diet ↓ Total IBS-SSS score in LGG vs. habitual diet</td>
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<tr>
<td>Eswaran et al., 2016</td>
<td>Randomised controlled trial</td>
<td>Standard IBS dietary advice</td>
<td>Dietary advice, dietitian-led</td>
<td>Rome III IBS-D LFD n=45 Standard diet n=39</td>
<td>4 weeks AR question BSFS 11-point rating scale for pain, bloating and urgency</td>
<td>No significant difference in AR between the groups ↑% patients achieved ≥30% reduction in pain in LFD vs. standard diet ↑ Improvement in pain, bloating, stool consistency, stool frequency and urgency in LFD vs. standard diet</td>
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<td><strong>Blinded, IBS</strong></td>
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<td>Böhn et al., 2016</td>
<td>Randomised, single-blind controlled trial</td>
<td>Standard IBS dietary advice</td>
<td>Dietary advice, dietitian-led</td>
<td>Rome III IBS LFD n=38 Standard diet n=37</td>
<td>4 weeks IBS-SSS BSFS</td>
<td>↓ Total IBS-SSS score in LFD and standard diet between week 0 and week 4 No difference between groups at week 4</td>
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<tr>
<td>Zahedi et al., 2017</td>
<td>Randomised, single-blind controlled trial</td>
<td>Standard IBS dietary advice</td>
<td>Dietary advice, dietitian-led</td>
<td>Rome III IBS-D LFD n=55 Standard diet n=55</td>
<td>6 weeks IBS-SSS BSFS</td>
<td>↓ Total IBS-SSS and stool frequency score in LFD and standard diet between week 0 and week 4 ↓ Total IBS-SSS in LFD vs. standard diet at end of trial</td>
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<tr>
<td>Reference</td>
<td>Trial design</td>
<td>Comparator</td>
<td>Diet delivery</td>
<td>Participants</td>
<td>Duration</td>
<td>Symptom assessment</td>
<td>Main findings at end of trial</td>
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<td>Halmos et al., 2014</td>
<td>Randomised, single-blind controlled crossover trial</td>
<td>Typical Australian diet</td>
<td>Feeding study, dietitian-led</td>
<td>Rome III IBS</td>
<td>21 days per diet, 21 days washout</td>
<td>100mm VAS for abdominal pain, bloating, flatulence, dissatisfaction with stool</td>
<td>↓ Overall GI symptom severity on LFD vs. Typical Australian diet; 70% patients on LFD had &gt;10mm reduction in overall GI symptom severity</td>
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<tr>
<td>McIntosh et al., 2016</td>
<td>Randomised, single-blind controlled trial</td>
<td>High FODMAP diet</td>
<td>Dietary advice, dietitian led</td>
<td>Rome III IBS</td>
<td>3 weeks</td>
<td>IBS-SSS</td>
<td>↓ IBS-SSS in LFD vs. baseline; ↓ urinary histamine in LFD; ↑ Adlercreutzia and Dorea in LFD</td>
</tr>
<tr>
<td>Staudacher et al., 2017</td>
<td>Randomised, single-blind controlled trial</td>
<td>Placebo 'sham' diet</td>
<td>Dietary advice, dietitian led</td>
<td>Rome III IBS-D, IBS-M, IBS-U LFD n=51 Sham n=53</td>
<td>4 weeks AR question BSFS IBS-SSS GSRS</td>
<td>No difference in AR between diets; ↓ Total IBS-SSS score in LFD vs. sham; ↑ % responders (50 point reduction IBS-SSS) in LFD vs. sham; ↓ Severity abdominal pain, overall symptoms in LFD vs. sham; ↓ Stool consistency in LFD vs. sham</td>
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LFD, low FODMAP diet; AR, adequate relief; LGG, Lactobacillus rhamnosus GG; IBS-SSS, IBS Severity Scoring System; VAS, visual analogue scale; GI, gastrointestinal; IBS-D, IBS diarrhoea subtype; IBS-M, IBS mixed subtype; IBS-U, IBS unsubtyped; BSFS, Bristol Stool Form Scale; GSRS, Gastrointestinal Symptom Rating Scale
Two studies to date have utilised existing dietary IBS treatments as controls to establish superiority or inferiority of the low FODMAP diet (Bohn et al., 2015, Eswaran et al., 2016). The first trial randomised patients with IBS to follow either low FODMAP dietary advice or standard IBS advice for 4 weeks (NICE, 2008, McKenzie et al., 2016, Bohn et al., 2015). GI symptom severity significantly improved on both diets with no difference between the groups at end of trial, suggesting equal efficacy (50% defined as responders following low FODMAP diet vs. 46% following standard IBS advice). However, the standard IBS advice also involved restriction of some high-FODMAP foods (e.g. onions, pulses, sugar-free chewing gum) therefore potentially limiting the comparative effect size of the low FODMAP diet. The second trial also randomised patients to follow low FODMAP dietary advice or standard IBS advice, but in contrast to the first trial, the standard IBS advice did not restrict any high-FODMAP foods. Therefore, the results of this trial likely represent the true effectiveness of the two dietary approaches. There was a similar proportion of ‘responders’ in both diet groups, however there were more pain responders in the low FODMAP diet group compared to the standard IBS advice group. Both trials are limited by the lack of placebo-control group.

One trial compared the low FODMAP diet to a probiotic, *Lactobacillus rhamnosus* GG (LGG) (Pedersen et al., 2014). Patients with IBS were randomised to one of three groups for 6 weeks; low FODMAP dietary advice, LGG, or to continue with a habitual diet. There was a significantly greater reduction in symptom severity score in the low FODMAP diet and LGG groups at end of trial compared to the habitual diet group, although there was a significant reduction in score in all three groups between baseline and end of trial (likely a result of the Hawthorne effect (Sedgwick and Greenwood, 2015). The results should, however, be interpreted with caution due to a number of limitations. The trial was unblinded and there was likely an uneven expectation bias across the three groups; the expectation of a positive effect in the two treatment groups and expectation of lack of benefit in the control group (Staudacher et al., 2017a). Furthermore, patients who discontinued the trial prematurely were excluded from the final analysis, and this analysis may not represent the true effectiveness of the treatments. The low FODMAP diet has also been compared to gut-directed hypnotherapy (Peters et al., 2016) and yoga (Schumann et al., 2018), both of which revealed similar efficacy of the control treatments compared to the low FODMAP diet.

Collectively, trials comparing the low FODMAP diet to active control groups generally indicate that other treatments may also improve GI symptoms in IBS and are a viable option for patients in whom the low FODMAP diet is not clinically indicated or in non-responders. However, all three trials lack a placebo-control group (Staudacher et al., 2017a).
Rather than comparing the low FODMAP diet to a no-treatment control group, a ‘typical’ FODMAP intake or an alternative treatment, a Canadian research group used a high FODMAP diet as the control treatment (McIntosh et al., 2016). Patients were randomised to one of two diets which were provided as dietary advice and were blinded to group allocation. Promisingly, end of trial symptom severity score was significantly lower in the low FODMAP compared to the high FODMAP diet group. However, the use of the high FODMAP diet control likely inflates the effect size of the low FODMAP diet since the high FODMAP diet could induce GI symptoms.

1.4.6 Impact of the low FODMAP diet on gut microbiota

Dietary patterns have been associated with GI microbiota profiles and with microbial enterotypes. A comparative study revealed that the GI microbiota of children from a rural African village was enriched in Bacteroidetes and Prevotella and low in Firmicutes, while that of European children was higher in Enterobacteriaceae (De Filippo et al., 2010). A subsequent analysis suggested that the GI microbiota of African children living in urban areas resembles that of European children, a microbiota adept at metabolising animal protein and fat rather than polysaccharides (De Filippo et al., 2017). A similar pattern was observed in a study of Filipino children living in a rural region compared to those living in an urban region, with the former exhibiting a Prevotella-dominant and the latter a Bacteroides-dominant microbiota (Nakayama et al., 2017). Similarly, healthy American volunteers with a predominantly animal-based diet displayed a Bacteroides enterotype, while a carbohydrate-rich diet associated with a Prevotella enterotype (Wu et al., 2011), although others have argued that the notion of enterotypes is an oversimplification of diet-associated GI microbiota profiles (De Filippis et al., 2016). Rapid changes in the GI microbiota were observed following low-fat/high-fibre diets and high-fat/low-fibre dietary interventions, although did not shift the enterotypes associated with long-term dietary patterns (Wu et al., 2011). A further study revealed that a short-term dietary intervention composed entirely of animal products rapidly increased the abundance of bile-tolerant bacteria (Alistipes, Bilophila, Bacteroides) while a plant-based diet increased Firmicutes adapted to metabolising carbohydrates (Roseburia, Eubacterium rectale, Ruminococcus bromii) (David et al., 2014). These studies utilise extreme dietary patterns (for example, based entirely upon animal or plant products) that are unlikely to reflect the actual habitual dietary intake of the majority of the population. Nonetheless, it is evident from these studies that long-term and short-term dietary patterns are associated with distinct microbial profiles and modification of dietary components, including carbohydrates and proteins, markedly alter these profiles.

A low FODMAP diet involves the manipulation of dietary carbohydrates, although fibre is not restricted and it should not be a low fibre diet. That said, some trials have also observed a lower overall fibre intake following the low FODMAP diet compared to a control diet (Bohn et al.,
Fructans and GOS are considered prebiotic carbohydrates and a low FODMAP diet could therefore be considered a prebiotic-restricted diet (Gibson et al., 2017). The change in dietary substrate is likely to alter the composition of the GI bacterial community (Sonnenburg and Sonnenburg, 2014). It is therefore unsurprising that changes in GI microbiota composition have been observed following a low FODMAP diet in patients with IBS.

In the first ever trial of low FODMAP dietary advice, patients with IBS were randomised to either the low FODMAP diet or to continue with their habitual diet for 4 weeks (Staudacher et al., 2012). Faecal samples were collected before and after 4 weeks of the diet. Total bacterial abundance and the relative and absolute abundance of several bacterial groups were quantified using FISH. Compared to patients with IBS following their habitual diet, those following low FODMAP dietary advice had lower relative abundance of faecal Bifidobacteria. None of the other measured bacterial groups were affected by the diet, including Faecalibacterium prausnitzii or Lactobacillus. Interestingly, the change in Bifidobacteria abundance in the low FODMAP diet group was negatively correlated with baseline abundance. Similarly, in healthy individuals, a lower baseline Bifidobacteria abundance is correlated with a greater increase in abundance following prebiotic supplementation (Rycroft et al., 2001). This was the first trial to demonstrate an effect of the low FODMAP diet on the GI microbiota, however there are thousands of other bacterial groups that were not captured in this analysis and which may be influenced by diet.

The aforementioned feeding study compared the effect of the low FODMAP diet to a typical Australian diet on the GI microbiota (Halmos et al., 2014a), with some discrepant findings compared to the previous trial. Quantitative polymerase chain reaction (qPCR) was used to quantify the absolute and relative abundance of a selection of bacterial groups at baseline and after each intervention period. The absolute abundance of total bacteria and most of the measured bacterial groups was significantly lower following the low FODMAP diet compared to the typical Australian diet. The relative abundance of Clostridium cluster XIVa was significantly greater on the typical Australian diet compared to both the low FODMAP diet and the habitual diet, although there was no difference between the low FODMAP diet and habitual diet.

Compared to the habitual diet, the absolute abundance of F. prausnitzii was significantly lower following the low FODMAP diet, whereas the relative abundance of Akkermansia muciniphila was greater following the typical Australian diet compared to the habitual diet. Ruminococcus torques abundance was significantly greater on the low FODMAP diet compared to the typical Australian diet. The FODMAP content of the typical Australian diet was slightly higher than that of the habitual diet and this could have had a prebiotic effect. Furthermore, similar to the previous trial, the analysis was biased to the bacterial groups chosen for analysis.
Chapter 1: Introduction

A trial in Canada measured the effects of low FODMAP diet compared to high FODMAP dietary advice on the GI microbiota in patients with IBS (McIntosh et al., 2016). In contrast to the previously described trials, 16S rRNA sequencing was used to characterise the GI microbiota in faecal samples collected before and after the 3-week intervention. Bacterial richness was significantly greater following the low FODMAP diet. However, there was no difference in Bifidobacteria abundance between the diet groups at end of trial, although the high FODMAP diet appeared to have a prebiotic effect since there was an increase in Bifidobacteria abundance in this group during the trial. In addition, abundance of Adlercreutzia, a hydrogen-utilising bacterium, and Dorea were significantly greater following the low FODMAP diet compared to the high FODMAP diet. Sequencing the 16S rRNA gene permits a comprehensive analysis of all of the GI bacteria, rather than just species of interest, thus having the potential to capture unanticipated effects of the diet (Morgan and Huttenhower, 2012). However, it provides no information on the functions of the microbiota.

Finally, a large placebo-controlled trial compared low FODMAP to placebo sham dietary advice in IBS and a stool sample was collected prior to and following the intervention (Staudacher et al., 2017b). Similar to the trial above, 16S rRNA sequencing was used to characterise the microbiota and showed significantly lower Bifidobacteria abundance following the low FODMAP diet compared to the sham diet, with no effect on alpha diversity.

All the trials to investigate the effect of a low FODMAP diet on GI microbiota have been of 3-4 weeks’ duration and the longer-term effects require investigation since the use of a low FODMAP diet exceeding 4 weeks is common in clinical practice. Furthermore, the effect of FODMAP reintroduction and possible reversal of the microbiota changes should be explored.

1.4.7 Impact of the low FODMAP diet on nutrient intake

The low FODMAP diet requires many dietary modifications and restricts foods from several food groups, therefore it can have an impact upon nutrient intakes. The nutrients most likely to be compromised during the low FODMAP diet are calcium (due to the restriction of lactose in dairy products), iron (due to the restriction of iron-fortified cereals) and fibre (due to the restriction of certain grains and certain fruits and vegetables). Additionally, overall energy intake may be compromised, particularly considering the restriction of staple foods such as bread and pasta.

Trials in which patients are instructed to incorporate low FODMAP dietary advice into the habitual diets are more representative of the effect of the diet on nutrient intakes as opposed to trials in which all food is provided to participants. Unfortunately, not all trials of the low FODMAP diet have presented a comprehensive nutritional analysis, often omitting...
micronutrients (Bohn et al., 2015, Eswaran et al., 2016) or entirely omitting nutrient and FODMAP intakes (McIntosh et al., 2016, Pedersen et al., 2014, Pedersen et al., 2017).

Compared to patients continuing to follow a habitual diet, patients with IBS following low FODMAP dietary advice had lower calcium intakes after 4 weeks (Staudacher et al., 2012). A further trial showed that compared to patients following traditional IBS advice, those following low FODMAP dietary advice had significantly lower energy intake, as well as lower carbohydrate and fibre intakes (Bohn et al., 2015). In a recent trial of 95 patients with IBS, calcium intake was not different between the low FODMAP and sham diet groups at end of trial, although fewer patients at end of trial reached the calcium dietary reference value (DRV) compared to baseline in the low FODMAP diet group (Staudacher et al., 2015).

The long-term effects of the low FODMAP diet on nutrient intakes were investigated in a study of patients 6-18 months after receiving FODMAP reintroduction advice (O’Keeffe et al., 2018). At the long-term follow-up, 82% continued to follow an ‘adapted FODMAP’ diet, while 18% had returned to the habitual diet. In the adapted FODMAP group, total FODMAP intakes were significantly lower while nutrient intakes were similar to the habitual diet group. Another study showed that fibre intake declined on the low FODMAP diet but returned to normal following FODMAP reintroduction (Harvie et al., 2017). These findings suggest that FODMAP reintroduction may resolve nutritional inadequacies occurring with the low FODMAP diet, however both studies used a food frequency questionnaire to estimate nutrient and FODMAP intakes, which lacks accuracy compared to prospective food records (Bingham et al., 2007, Prentice et al., 2011).

1.4.8 Evidence for the low FODMAP diet in IBD

There is evidence supporting the effectiveness of the low FODMAP diet in IBS, including a large placebo-controlled trial (Staudacher et al., 2017b). Since a large proportion of patients with inactive IBD continue to experience FGS (Halpin and Ford, 2012), it is unsurprising that interest in the low FODMAP diet has grown in recent years. Indeed, the first RCT in this patient population has been published recently (Pedersen et al., 2017). Prior to this, two uncontrolled studies had indicated the potential effectiveness of the diet in IBD (Gearry et al., 2009a, Prince et al., 2016).

An Australian retrospective study investigated the effect of low FODMAP dietary advice provided to patients with IBD as part of routine clinical practice (Gearry et al., 2009a). Patients who had received low FODMAP dietary advice were telephoned up to 18 months later and a telephone questionnaire, regarding the advice provided, symptom responses to dietary changes, and adherence to the advice, was administered. Following the low FODMAP diet, most
GI symptoms had reportedly improved in both CD and UC. However, this study was uncontrolled, meaning that the magnitude of placebo response to the diet cannot be estimated. Furthermore, due to the retrospective nature, changes in disease activity, medication and other dietary components during the low FODMAP diet cannot be accurately assessed.

The second study was a prospective uncontrolled study of low FODMAP dietary advice performed by our group to a UK population as part of routine clinical practice in a dietetic gastroenterology outpatient clinic (Prince et al., 2016). Following the advice, 78% of patients reported adequate relief of FGS compared to 16% at baseline, in addition to a large proportion experiencing an improvement in individual FGS such as abdominal pain, bloating and flatulence following the advice. This study was uncontrolled and, hence, carries the same limitation as the aforementioned retrospective study. The results of this study may also be subject to referral and selection bias and responder bias, as well as potential confounding variables that cannot be accounted for, such as changes in disease activity and medications during the diet.

The first RCT of the low FODMAP diet in IBD was conducted in Denmark and recruited 89 patients with inactive or mild-to-moderate IBD (Pedersen et al., 2017). Participants were randomised to either low FODMAP dietary advice or to continue habitual dietary intake for 6 weeks. Functional GI symptoms and health-related quality of life were evaluated before and after the diet. The primary outcome was the proportion of patients classified as ‘responders’ to the low FODMAP diet, defined as at least a 50-point reduction in total IBS Severity Scoring System score between baseline and end of trial. Significantly more patients responded to the intervention in the low FODMAP diet (81%) compared to the control group (46%) (P<0.01), indicating a large placebo response considering that participants in the control group received no intervention. This may represent the fluctuating nature of FGS or the placebo response induced through inclusion in a trial, the Hawthorne effect (Sedgwick and Greenwood, 2015). Health-related quality of life was significantly greater in the low FODMAP diet group than the control group at end of trial, in line with the improvement in GI symptoms (Gracie et al., 2017).

There are some noteworthy limitations to this trial. Firstly, the control group were not a placebo intervention or an active comparator, and therefore the trial was unblinded. The lack of placebo dietary advice renders it impossible to account for the placebo response, although the response rate in the control group was surprisingly high. Secondly, although the primary outcome was analysed based on intention to treat, all other outcomes were analysed per protocol. The findings of the secondary outcomes therefore do not represent the effectiveness of the diet in clinical practice, although they do demonstrate efficacy when the diet is followed optimally. Thirdly, a food frequency questionnaire was used to measure baseline dietary intake in all participants, which correlates poorly with the more robust prospective food record (Key et al.,
Furthermore, since dietary intake was not measured at follow-up, intake of FODMAPs and other potentially clinically relevant dietary components (e.g. fibre) during the trial cannot be established. Finally, clinical disease activity indices were used alone to determine IBD activity for trial inclusion. Although these correlate well with disease activity and avoid more invasive measures such as endoscopy, the value of symptom-based measures in the context of functional symptoms is questionable (Quigley, 2016). Patients experiencing FGS may be incorrectly classified as having active IBD as a result of FGS elevating disease activity scores, and hence incorrect inferences may be made in analysis, for example when performing sub-group analyses by disease activity. Thus, it is likely that a combination of active and inactive patients were included in this trial.

A placebo-controlled trial of the low FODMAP diet is warranted. However, it would still be unclear whether fermentable carbohydrates are responsible for FGS in IBD, and a randomised, placebo-controlled re-challenge trial using pure fermentable carbohydrates, as has been conducted in IBS, would help to address this (Shepherd et al., 2008).

### 1.4.9 Concerns surrounding the low FODMAP diet in IBD

The low FODMAP diet is gaining considerable interest in IBD with the emergence of studies suggesting it may be effective for the management of IBS-like symptoms (Prince et al., 2016, Gearry et al., 2009a, Pedersen et al., 2017). There are two major concerns regarding the use of the low FODMAP diet in IBD.

The first concern relates to the theoretical detrimental effect of restricting dietary prebiotic carbohydrates during the low FODMAP diet and the associated decline in immune-modulatory bacteria. The low FODMAP diet paradoxically improves GI symptoms while causing a decline in numerous bacteria, in particular Bifidobacteria, but also *Faecalibacterium prausnitzii* and *Clostridium cluster XIVa*. These immune-modulatory bacteria can interact with the GI immune system through pattern-recognition receptors on host epithelial and immune cells, and this in turn may influence the enteric nervous system and the central nervous system. Furthermore, Bifidobacteria abundance correlates with number of days of abdominal pain in patients with IBS, suggesting an association between this bacterium and GI symptoms (Parkes et al., 2008).

Bacterial dysbiosis is present in IBD, generally characterised by a decline in Firmicutes, especially *F. prausnitzii*, and an increase in Proteobacteria, especially *Escherichia coli* in CD (Kostic et al., 2014). Although it is unclear whether this is a cause or consequence of inflammation, there is evidence that altered microbiota may play a role in the generation of GI inflammation (Kolho et al., 2015, Prosberg et al., 2016, Rooks et al., 2014, Sokol et al., 2008). Moreover, several studies have revealed reduced Bifidobacteria abundance in IBD, although this finding is not consistent
Bifidobacteria are known to have anti-inflammatory effects *in vitro* and *in vivo* (Riedel et al., 2006, Sarkar and Mandal, 2016, Fanning et al., 2012, Turroni et al., 2016, Gibson and Wang, 1994, Picard et al., 2005). Fructan supplementation, effectively representing the opposite intervention to fructan restriction on the low FODMAP diet, increases faecal Bifidobacteria and *F. prausnitzii* abundance in healthy volunteers (Ramirez-Farias et al., 2009). Conversely, fructan supplementation had no effect on faecal microbiota in active CD but lamina propria immune cell phenotype was altered, with reduced pro-inflammatory IL-6-positive DC and higher anti-inflammatory IL-10-positive DC (Benjamin et al., 2011b). Furthermore, inulin-enriched oligofructose supplementation resulted in reduced faecal calprotectin in patients with UC in a small group of patients, and therefore restricting these prebiotic carbohydrates in the diet may increase faecal calprotectin (Casellas et al., 2007). Therefore, reducing dietary prebiotics could exacerbate the existing dysbiosis in IBD and may even alter GI immune cell function irrespective of microbiota alterations. These changes could, in turn, impact upon intestinal inflammation and IBD course. The effect of the low FODMAP diet on GI microbiota and immune function in IBD should therefore be investigated prior to widespread implementation of the diet.

In a low FODMAP diet feeding study, nine patients with CD were also recruited and subjected to the same study protocol as the patients with IBS (Halmos et al., 2016). The microbiota were altered in IBD in a similar fashion to the patients with IBS. In contrast to the other trials assessing the GI microbiota following the low FODMAP diet in IBS (Staudacher et al., 2017b, Staudacher et al., 2012), relative abundance of Bifidobacteria was not altered in patients with IBS or CD following the low FODMAP diet, although absolute abundance was reduced. Although the trial was not powered to detect differences in patients with CD and these patients represented a small proportion of the total sample size, this provides preliminary evidence that the low FODMAP diet may detrimentally impact upon immune-modulatory bacteria in IBD.

The second concern regarding the use of the low FODMAP diet in IBD relates to nutritional adequacy. As discussed above, the low FODMAP diet may compromise intakes of macronutrients and micronutrients, although nutritional adequacy may be restored following FODMAP reintroduction. Patients with IBD are at heightened risk of malnutrition resulting from the effects of chronic GI inflammation on food intake, nutrient absorption and metabolism (Gerasimidis et al., 2011). Owing to the elevated risk of osteoporosis and anaemia in IBD, it is particularly important that calcium and iron intakes are adequate (Lomer, 2011). Reduced energy intake could exacerbate existing malnutrition and reduced fibre intake could have clinical implications since lower fibre consumption has been associated with a greater risk of CD relapse (Brotherton et al., 2016). Therefore, all the ‘at risk’ nutrients on a low FODMAP diet are
especially important in IBD. Particular care should be taken to ensure that excluded foods are replaced with nutritionally equivalent alternatives and future research should establish the effect of the low FODMAP diet on nutrient intakes in IBD.

1.5 Conclusion and future research

Many patients with IBD report persistent GI symptoms in the absence of GI inflammation, which may detrimentally impact upon HR-QOL. Scarce evidence exists on the management of these common and debilitating FGS in IBD. The most promising approach is the modification of dietary fermentable carbohydrates (the low FODMAP diet), which improves GI symptoms in 50-80% of patients with IBS. Little evidence exists on FODMAP intakes in patients with IBD, with and without FGS, although one case-control study revealed lower fructan intakes in active IBD compared to healthy controls. Two uncontrolled studies and a non-placebo controlled trial indicate potential effectiveness of the low FODMAP diet for FGS in IBD. However, it is unclear from these studies whether fermentable carbohydrates per se are responsible for the improvement in FGS. This challenge can be overcome by using a FODMAP restriction and re-challenge approach. These studies also do not account for the placebo response that can be considerable in IBS and IBD. Additionally, concerns regarding the consequences of the microbiota alterations on immunology, as well as the effect of the low FODMAP diet on nutritional adequacy in IBD, warrant a placebo-controlled trial to confirm both the effectiveness and safety of the diet in IBD.

1.6 Aims of thesis

The aim of this thesis was to explore the role of fermentable carbohydrates in FGS in IBD using a variety of methodological approaches. This was achieved by addressing the following aims:

1. To conduct a case-control study to investigate FODMAP, nutrient and fibre intakes in patients with active IBD (inflammatory GI symptoms), inactive IBD with FGS (functional GI symptoms), inactive IBD without FGS (no GI symptoms) and healthy controls (no GI symptoms).
2. To conduct a randomised, double-blinded, placebo-controlled, crossover, re-challenge trial to determine whether individual fermentable carbohydrates exacerbate FGS in patients with inactive IBD.
3. To conduct a randomised controlled trial to investigate the effect of low FODMAP dietary advice compared to placebo ‘sham’ dietary advice on FGS, GI microbiota, circulating T-cell gut-homing phenotype and markers of intestinal inflammation in patients with inactive IBD with FGS.
2 FODMAP and nutrient intake in inflammatory bowel disease and the association with functional gastrointestinal symptoms
2.1 Introduction

The low FODMAP diet is a potential therapy for common FGS in patients with inactive IBD (Gearry et al., 2009a, Prince et al., 2016, Pedersen et al., 2017). It is unclear whether patients with IBD intentionally or unintentionally manipulate dietary FODMAP intake for the management of GI symptoms. Data on FODMAP intakes in IBD are limited to one case-control study in which a validated food-frequency questionnaire (FFQ) (Dunn et al., 2011) was used to demonstrate that fructan intakes were significantly lower in active CD compared to inactive IBD and healthy controls (Anderson et al., 2015). However, energy intakes were not estimated, making it impossible to attribute lower fructan intakes to avoidance of fructans specifically rather than to a reduced overall food intake. Indeed, lower energy intake in children, adolescents and adults with IBD compared to healthy controls has been observed (Hartman et al., 2016, Benjamin et al., 2011a). Nonetheless, reduced fructan intake in active IBD could relate to dietary restrictions commonly practiced for symptom control or due to perceived risk of relapse associated with certain foods (Prince et al., 2011, Limdi et al., 2016). Furthermore, intakes of FODMAPs besides fructans could be influenced by restrictions commonly observed in IBD, including dairy products (lactose), beans (galacto-oligosaccharides; GOS) and certain fruits and vegetables (fructose, polyols) (Vagianos et al., 2016, Zallot et al., 2013).

There remains a major knowledge gap regarding fructan intakes in UC, as well as the intakes of other FODMAPs, in both CD and UC. It is also unclear whether FODMAP restriction relates to active IBD per se, or due to GI symptoms of any aetiology. Investigating FODMAP intakes in patients with active IBD (inflammatory GI symptoms), inactive IBD who have concurrent FGS (functional GI symptoms) and inactive IBD without FGS (no GI symptoms) would help delineate these behaviours. Establishing whether IBD patients with GI symptoms specifically avoid foods high in FODMAPs despite being naïve to a low FODMAP diet would lend further support to FODMAPs as inducers of GI symptoms and is an important consideration since the long-term microbial and clinical implications of chronic FODMAP restriction are unknown.

Previous studies have also reported a range of nutrient inadequacies in IBD, including vitamin D, calcium, iron and B vitamin intakes (Vagianos et al., 2007, Vidarsdottir et al., 2016, Filippi et al., 2006), and evidence exists of altered macronutrient distribution (Geerling et al., 2000). However, studies have generally featured small sample sizes, limited dietary assessment methods, heterogeneity in patients recruited, inconsistent nutrient reporting and the lack of a non-IBD control group (Vagianos et al., 2007, Vidarsdottir et al., 2016). Therefore, a comprehensive assessment of nutrient intakes using a robust dietary assessment method in a large group of patients with active and inactive CD and UC, and healthy controls, is warranted.
2.2 Aims and objectives of the case-control study

The aim of this case-control study was to investigate the effect of GI symptoms on FODMAP, nutrient and fibre intakes in patients with active IBD (inflammatory GI symptoms), inactive IBD with FGS (functional GI symptoms), inactive IBD without FGS (no GI symptoms) and healthy controls (no GI symptoms).

Primary objective: to compare fructan intakes in patients with active IBD, inactive IBD with FGS, inactive IBD without FGS and healthy controls

Secondary objectives: in patients with active IBD, inactive IBD with FGS, inactive IBD without FGS and healthy controls:

1. To compare GOS, lactose, fructose and polyol intakes
2. To compare energy, macronutrient, micronutrient and fibre intakes
3. To compare food-related quality of life
2.3 Hypotheses

Null (H0) hypothesis:
There is no difference in FODMAP, nutrient and fibre intakes or food-related quality of life in patients with active IBD, inactive IBD with FGS, inactive IBD without FGS and healthy controls.

Alternative hypothesis:
There is a difference in FODMAP, nutrient and fibre intakes or food-related quality of life in patients with active IBD, inactive IBD with FGS, inactive IBD without FGS and healthy controls.

2.4 Methodology

2.4.1 Study design
This was a 7-day case-control study of dietary intake in patients with IBD and healthy controls. This duration of food diary was chosen, rather than the typical 3-day or 4-day food diary (Vidarsdottir et al., 2016, Vagianos et al., 2007, Filippi et al., 2006, Hartman et al., 2016), to control for inter-diurnal variation in eating behaviour and to improve precision of global food intake assessment. It was felt that a duration exceeding 7 days would compromise the quality of diary completion and rates of diary return. Although a 7-day diary could theoretically induce participant fatigue and compromise completion rates compared to a 3-day or 4-day diary, a 7-day food diary accounts for a change in dietary habits and inaccurate recording that may occur upon beginning a food diary and the fatigue that may occur towards the end, leaving several days in the middle of the diary that may more accurately represent dietary intake (Biltoft-Jensen et al., 2009).

2.4.2 Study sites
Patients with IBD were recruited from gastroenterology clinics at Guy’s and St Thomas’ NHS Foundation Trust, Barts Health NHS Trust and King’s College Hospital NHS Foundation Trust, London, UK between September 2016 and March 2018. Visits were conducted either in the hospital clinic or in the Department of Nutritional Sciences, King’s College London. All participants were recruited by the author of this thesis (SC), except for three patients with active UC who provided a baseline food diary for a clinical trial and consented to food diary data being used in subsequent studies. Diaries from patients with inactive IBD with FGS were baseline diaries collected as part of the RCT described in Chapters 4-6 of this thesis. These diaries were identical and completed to the same level of detail as the other study groups.
2.4.3 Patient selection, inclusion and exclusion criteria

Inclusion and exclusion criteria for patients with active IBD, inactive IBD with FGS, inactive IBD without FGS, and healthy controls was different and the criteria for each group are detailed in Table 2.1. Justification for the use of the Harvey-Bradshaw Index (HBI) for CD activity is described in detail in section 4.4.2. The Simple Clinical Colitis Activity Index (SCCAI) was chosen as a non-invasive measure of UC activity that appears to be the strongest of a range of non-invasive indices in reflecting UC activity (Walsh et al., 2014, Turner et al., Higgins et al., 2005).

The HBI thresholds for active (HBI ≤3) and inactive CD (HBI ≥5) were chosen as those showing good correlation with the Crohn’s Disease Activity Index (CDAI) thresholds for active and inactive CD (Vermeire et al., 2010, Foti et al., 2015, Falvey et al., 2015). The SCCAI thresholds for active (SCCAI ≥4) and inactive UC (SCCAI ≤2) were chosen in light of evidence that these reflect disease activity with optimal sensitivity and specificity (Walsh et al., 2016, Marin-Jimenez et al., 2016, Walsh et al., 2014). Due to issues of accuracy of the scoring systems in detecting active and inactive disease, there was a risk of active patients being in the inactive group, and vice versa. Therefore, to create a clear distinction between patients with active and inactive IBD, patients on the borderline of the cut offs for these scores (HBI 4, SCCAI 3) were not eligible for the study. For the same reasons, an objective marker of the presence or absence of inflammation (CRP, calprotectin imaging) was also required to define active or inactive disease, respectively.

The HBI and SCCAI could not be used to establish inactive IBD in patients with FGS as the presence of FGS could elevate scores despite the absence of GI inflammation (and thus, would incorrectly exclude from the study or incorrectly allocate to the active IBD group), a flaw with symptom-based diseases activity indices which has been highlighted previously (Gracie et al., 2017). Therefore, inactive IBD was established in this group using inflammatory markers (CRP and/or faecal calprotectin). In a large proportion of patients, only a recent serum CRP concentration was available, since this is routinely collected in clinic. While a high serum CRP indicates active IBD (in combination with clinical indices), a low CRP may be present in both active and inactive IBD as this marker has poor sensitivity for identifying disease activity (Mosli et al., 2015). However, a low HBI or SCCAI in addition to a low CRP increases the likelihood of inactive disease.

Patients currently taking medications for IBD (5-ASA, thiopurines, biologics, steroids) were eligible, however patients with changes in medication in the 2 weeks preceding screening were excluded, in order to prevent any effects of medication dose changes on disease activity, with a subsequent effect on dietary intake. Patients with current stricturing CD were excluded from all IBD groups since it was assumed that the presence of a stricture could lead to a change in dietary
intake distinct from inflammatory GI symptoms or FGS. However, patients with a history of stricturing CD were eligible, but those with a current stricture were not due to the potential impact of that on current intake. With similar rationale, patients with a current stoma or with short bowel syndrome, as well as those currently following a special diet (e.g. Paleolithic diet, gluten-free diet) were excluded. Patients implementing changes in IBD medications in the preceding 2 weeks were excluded to prevent confounding associated with drug side-effects. Patients with other major co-morbidities were assessed on a case-by-case basis. Conditions judged to have a potential impact on dietary intake (such as diabetes and coeliac disease) were excluded.
### Table 2.1 Inclusion and exclusion criteria for the different groups of the case-control study

<table>
<thead>
<tr>
<th>Group</th>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
</tr>
</thead>
</table>
| Active IBD (inflammatory GI symptoms) | Standard inclusion criteria:  
- Aged 18-75 years old  
- Able to give informed consent  
- IBD (CD or UC) diagnosed by standard clinical, histological and radiological criteria  
- Diagnosis of IBD ≥3 months  

  Standard inclusion criteria plus, active IBD as defined by:  
- A subjective measure of active disease: HBI score ≥5 for CD, or SCCAI ≥4 for UC  
- An objective measure of active disease: CRP >10 mg/L and/or faecal calprotectin >250 µg/g and/or endoscopic or imaging investigations (in the last 4 weeks). | Standard exclusion criteria  
- Current stricturing CD  
- Extensive intestinal resection indicating short bowel syndrome  
- Current stoma  
- Changes in dose or initiation of the following in the past 2 weeks: immunosuppressant or biological therapy, oral 5-ASA or rectal preparations  
- Gastrointestinal surgery thought to be imminent  
- Other gastrointestinal disorders  
- Significant comorbidities (including hepatic, renal, cardiovascular, endocrine, respiratory or neurological)  
- Diabetes mellitus  
- Patients following a special or restrictive diet other than specifically IBD-related diets  
- Pregnant or lactating  
- Lack of ability to give informed consent |
| Inactive IBD with FGS (functional GI symptoms) | Standard inclusion criteria (as list for active IBD), plus inactive IBD as defined by:  
- A subjective measure of FGS: experiencing symptoms meeting the Rome III criteria for IBS-D, IBS-M, IBS-U, functional bloating or functional diarrhoea  
- An objective measure of inactive disease: CRP <10 mg/L and/or faecal calprotectin <250 µg/g and/or recent endoscopic evidence of inactive disease (last 4/52) | Standard exclusion criteria (as listed above for active IBD) |
| Inactive IBD without FGS (no GI symptoms) | Standard inclusion criteria (as list for active IBD), plus inactive IBD as defined by:  
- A subjective measure of inactive disease: HBI score ≤3 for CD, or SCCAI ≤2 for UC | Standard exclusion criteria (as listed above for active IBD) plus: Experiencing GI symptoms meeting the Rome III criteria for IBS, functional bloating or functional diarrhoea |
### Chapter 2: Case-control study of dietary intake in IBD

#### An objective measure of inactive disease: CRP <10 mg/L and/or faecal calprotectin <250 μg/g and/or endoscopic or imaging investigations (in the last 4 weeks).

<table>
<thead>
<tr>
<th>Healthy controls (no GI symptoms)</th>
<th>IBD or other gastrointestinal disorder (including any functional bowel disorder or coeliac disease)</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Aged 18-75 y</td>
<td>• Experiencing gastrointestinal symptoms meeting the Rome III criteria for IBS, functional bloating or functional diarrhoea</td>
</tr>
<tr>
<td>• Able to give informed consent</td>
<td>• Previous gastrointestinal resection</td>
</tr>
<tr>
<td></td>
<td>• Significant comorbidities (including hepatic, renal, cardiovascular, endocrine, respiratory or neurological)</td>
</tr>
<tr>
<td></td>
<td>• Diabetes mellitus</td>
</tr>
<tr>
<td></td>
<td>• Patients following a special or restrictive diet</td>
</tr>
<tr>
<td></td>
<td>• Pregnant or lactating</td>
</tr>
<tr>
<td></td>
<td>• Lack of ability to give informed consent</td>
</tr>
</tbody>
</table>

FGS, functional gastrointestinal symptoms; HBI, Harvey Bradshaw Index; SCCAI, Simple Clinical Colitis Activity Index; CRP, C-reactive protein; 5-ASA, 5-aminosalicylic acid; IBS-D, irritable bowel syndrome diarrhoea subtype; IBS-A, irritable bowel syndrome mixed subtype; IBS-U, irritable bowel syndrome unsubttyped
2.4.4 Ethics approval

Ethics approval was granted for the study by London – City and East Research Ethics Committee on 17th May 2016 (16/LO/0976). Health Research Authority approval was granted on 22nd July 2016. Local approvals were granted shortly thereafter. King’s College Hospital was opened as a new site after study initiation in November 2017, to enhance recruitment.

2.4.5 Trial protocol and procedures

2.4.5.1 Patients with IBD

Most patients were recruited to the study in gastroenterology clinics. Patients were either identified by the researcher prior to appointments with the gastroenterology team, or were referred to the researcher by gastroenterologists, nurses and pharmacists after consultations. Some patients were also identified in IBD infusion units, which were attended regularly by the researcher.

Patients were screened against inclusion and exclusion criteria in Table 2.1 and those meeting the eligibility criteria were provided with a full description of the study by the researcher and a participant information sheet (PIS). Patients were offered as long as they needed to consider participation. Individuals fulfilling all the eligibility criteria were invited to attend a 20-minute study visit.

2.4.5.2 Healthy controls

Healthy controls were identified through fortnightly circular emails to staff and students at King’s College London and Guy’s and St Thomas’ NHS Foundation Trust (GSTT). These were selected as they would likely be similar in geographical reach as the IBD patients.

The advertisement at King’s College London was included on four emails at different times of the year. The advertisement at GSTT was included on two news bulletin emails several months apart. To limit sampling bias inevitable from recruiting students (younger, higher educational status) and academic and clinical staff (higher socio-economic status, higher educational status), attempts were made to recruit from a diverse staff population including from emailing and posting leaflets to professional services staff and building maintenance staff.

Individuals contacting the researcher via email were screened against the eligibility criteria in Table 2.1 and were sent the PIS and were offered as long as they needed to consider
participation. Individuals fulfilling all the eligibility criteria were invited to attend a 20-minute study visit.

### 2.4.6 Protocol for visit (patients with IBD and healthy controls)

During the study visit, the information contained within the PIS was reiterated and participants provided informed consent. Once participants had provided informed consent, demographic information was gathered (age, ethnicity, education level, smoking status), as well as information on any current medications. The researcher then provided participants with a 7-day food diary to complete consecutively for the following 7-days. If patients planned holidays in the next few days or other circumstances likely to interfere with diary completion, they were asked to delay recording the diary until the next possible 7-day period in which they were carrying out normal activities. For the same reason, patients were not recruited between the 15th December and the 1st January, since dietary habits often deviate from normal during this period and diary completion would likely be poorer.

Patients with IBD were provided with three questionnaires for the measurement of Food-related Quality of Life (FR-QOL) and patient-perceived control of IBD and completion was permitted at any time during the food diary completion week. Healthy controls were also provided with a version of the FR-QOL questionnaire worded and validated for healthy individuals, and the instructions for completion were identical to those provided to patients with IBD.

Between three to four days into the recording period, participants were contacted by the researcher to provide an opportunity to ask any further questions and to address any concerns in completing the food diary and questionnaires.

Participants returned the food diary and questionnaires either in person or via post (e.g. patients living outside of London, work/study during office hours). Those returning via post were provided with a stamped addressed envelope. All diaries were checked for completeness and detail by the researcher (SC, thesis author) and any outstanding information required was gathered by direct questioning (those returning in person) or via telephone or email (returning in person or by post).

### 2.4.7 Energy reporting

Studies involving self-reported dietary intake are subject to misreporting energy and nutrient intakes, which may result from conscious or unconscious omission of foods and misreporting
portion sizes (Black, 2000a). This phenomenon has been established by comparing reported energy intake with the energy required to maintain body weight in long-term studies and by comparing reported energy intake with energy expenditure measured using doubly-labelled water (Lichtman et al., 1992, Black et al., 1993). This common problem in dietary assessment may result in under-estimation (or indeed over-estimation) of nutrient intakes and may even lead to spurious conclusions, particularly where under-reporting is more prevalent in one study group. Excluding under-reporters from analyses has been shown to alter study outcomes (Stallone et al., 1997).

A number of methods exist for estimating the extent of energy and nutrient misreporting. The Goldberg method calculates the likelihood of misreporting using the agreement between the ratio of energy intake (EI) to basal metabolic rate (BMR) and the ratio of energy expenditure to physical activity level (PAL). This method has limitations including under-estimating the proportion of under-reporters and only identifying individuals at the extremes of misreporting (Black, 2000b). A similar approach is to calculate the ratio of EI to the estimated energy requirement (EER), which has an expected value of 1. Several cut-offs for under-reporters, acceptable reporters and over-reporters have been proposed, including <0.78, 0.78-1.22, and >1.22 (Huang et al., 2005). Similar cut-offs have been used by other authors; for example, classifying acceptable reporters as falling between 0.74 and 1.28 (Black, 2000b).

In this study, EER was calculated by multiplying the BMR (Henry, 2005) by a PAL to calculate EER. Since occupational and exercise activity levels were not available for the participants, a PAL of 1.4 was applied for all participants, relating to a sedentary job (i.e. office work) and no regular exercise. This was based on evidence that less than half of the UK population meet current physical activity guidelines, and therefore the PAL of 1.4 provides a more conservative estimate or activity levels and to avoid over-estimating the prevalence of under-reporting (DoH, 2011, Black, 2000b). The ratio of EI to EER was calculated and patients were classified as either under-reporter (ratio below 0.78), 'acceptable' (ratio between 0.78-1.22), or over-reporter (ratio above 1.22).

However, under-reporters and over-reporters were not excluded from the intake analysis because the presence of IBD may cause a true deviation from a EI/EER of 1.0 not related to under- or over-reporting. There are two reasons for this. Firstly, actual BMR may be elevated in those with active IBD and thus calculated BMR and EER are likely to be under-estimated. Secondly, EI may legitimately be lower than EER in those with IBD due to pain, GI symptoms
and psychological state (Sousa Guerreiro et al., 2007, Benjamin et al., 2011a). Thus, a EI/EER deviating from 1.0 may simply be a manifestation of IBD.

### 2.4.8 Outcome measures and rationale: dietary assessment

#### 2.4.8.1 Food diaries

A 7-d prospective food diary was used to measure dietary intake in this study. Dietary intake can be measured retrospectively or prospectively. Retrospective methods include 24-hour diet recall, which captures recent dietary intake and does not rely on respondent literacy, and food-frequency questionnaires (FFQ), which are quick and inexpensive and can capture longer term dietary intake. However, 24-hour recall may not reflect usual intake and requires a skilled interviewer. Likewise, FFQ pose issues relating to the number of included food items and potential recall bias. Prospective methods overcome some of the limitations associated with retrospectively assessing dietary intake, for example eliminating recall bias, although these methods may induce unwanted behaviour change. Weighed food diaries are considered the gold standard dietary assessment method, but are burdensome to the respondent and require the equipment and skills to weigh all food consumed. Estimated (unweighed) food diaries are generally less cumbersome to the respondent, have shown to be comparable to weighed food diaries, and correlate more strongly with energy and protein biomarkers than FFQ (Bingham et al., 2007, Prentice et al., 2011). Portion-size estimation aids such as food models or food photographs may improve the accuracy of portion-size estimation in unweighed food diaries (Nelson et al., 1996).

The food record used in this study (Appendices 8.14 and 8.15) was based upon that used in an RCT conducted by a previous PhD student, and was originally developed based upon the National Diet and Nutrition Survey (NDNS) diary. This includes an example food diary page to demonstrate the level of detail required in the diary, and food photographs to aid in portion size estimation. The seven days of the diary were coded in different colours to facilitate easier navigation through the diary.

#### 2.4.8.2 Food diary administration

Detailed instruction on completing the food diary was provided to participants at the study visit. Participants were advised to improve accuracy of food records through the following measures:
1. Record all meals, snacks and drinks (including water) between getting up and going to bed on each diary day.

2. Take the diary to work or other activities and record dietary intake prospectively to minimise recall bias. Failing this, participants were to make a note of dietary intake on a phone, computer or notepad.

3. Provide brand name information for as many foods as possible.

4. Use the food portion photographs, household measures (cups, tablespoons, teaspoons), number of units (e.g. 1 biscuit) or food packaging (e.g. weight) for portion sizes.

5. Describe the ingredients and quantities, the number of servings and the cooking method for home-cooked dishes (specifying dry or cooked weight).

6. For foods eaten outside the home, describe the food in detail, provide the brand or restaurant name, estimated portion size and how much was eaten.

7. Record vitamin, mineral and other nutritional supplements.

Diaries were reviewed by the researcher upon return. Where additional information was required, this was requested in person or over the phone depending upon the method of diary return.

2.4.8.3 Data input and analysis

Several software packages are available for dietary analysis, including Dietplan, WinDiet and Nutritics, all with merits and limitations. Nutritics, which is based upon McCance and Widdowson’s composition of foods integrated dataset (CoFID) (PHE, 2015) was used in the current study. The ability to export dietary reports in batch was a desirable feature given the number of participant diaries collected in this study. Finally, Nutritics is regularly updated with new foods based upon information provided by manufacturers or publications and is therefore more likely to contain a comprehensive and current list of foods.

Dietary data was entered by two coders, Charlotte Howard and Alice Wright (student dietitians, King’s College London). Dietary input protocols previously used in the research group were followed, adapted and expanded for the purposes of this study. The coders had previous training and experience in using Nutritics. Regular meetings were held with the author of this thesis (SC) to resolve any data queries.

A ‘hierarchy’ was established for determining portion sizes that were not clearly specified in the diary, to ensure consistency in data entry. The ‘demographic average’ portion in Nutritics
was preferably used, and if this was not available, the Food Standards Agency portion size
guide was consulted (FSA, 2008).

Foods were entered into Nutritics as the closest existing food. For example, ‘Cheerios’ would
be entered as ‘multigrain hoops’. Where no existing food adequately matched the food in
the diary, a new food was created with nutrient content adapted according to online
information for the product. Composite foods were added as new recipes, unless individual
foods could be sufficiently representative. For example, a cheese and ham sandwich was
added as: bread + cheddar + ham + butter, rather than creating a recipe.

Following data entry all food records were batch exported into Microsoft Excel and data
checking was performed. Daily (average of the 7-day diary) intakes of energy, carbohydrate,
protein, fat, fibre, calcium, iron and vitamin C falling outside the 2.5th or 97.5th percentile
ranges for age and gender-matched 2008/2009 and 2011/2012 NDNS data were checked
against the food diaries for potential errors in data entry. Outliers were additionally
identified through constructing boxplots of the daily intakes of the aforementioned nutrients
in SPSS. Food diaries of individuals with an average daily intake falling outside 1.5 times the
interquartile range were checked for potential errors in data entry.

2.4.8.4 FODMAP intake analysis

The aim of this case-control study was to estimate FODMAP and nutrient intakes. Researchers at Monash University have performed extensive analyses of the fermentable carbohydrate content of foods using high-performance liquid chromatography (Yao et al., 2014, Muir et al., 2007, Muir et al., 2009, Biesiekierski et al., 2011b). From this published data, FODMAPs have been incorporated into the FoodWorks database (Xyris Software, Australia), access to which is now available online. This database permits researchers to enter foods consumed and generate a report of total and individual fermentable carbohydrate intakes of each individual.

The FODMAP database was used to determine fermentable carbohydrate intakes of
participants at baseline and end of trial. All diaries were entered into the database by the
author of this thesis.

During data entry, the following assumptions and practices were followed for all diaries:

1. For composite foods where no recipe was provided by the participant, a typical
   recipe for this food was used to estimate quantities of each ingredient. Should the
same food appear in a subsequent diary with no recipe provided, the same recipe was assumed. A popular UK recipe website was used consistently.

2. Where an exact match for a particular food was not available in the FODMAP database (largely due to the differences in UK and Australian dietary practices), the closest matching food was chosen. For example, ‘malted wheat breakfast cereal’ was entered as ‘plain wheat-based cereal’ in the FODMAP database.

3. Conversions were made for differences in cooked/uncooked foods between the diaries and the calculator. For example, if the diary stated ‘broccoli, boiled’ but the calculator contained only raw broccoli, the weight of broccoli entered was reduced by an appropriate factor, as outlined by the McCance and Widdowson’s Composition of Foods.

4. Foods devoid of carbohydrates (and therefore devoid of FODMAPs), such as plain eggs, meat and fish, are not included in the calculator and were not entered. Added ingredients that may contain FODMAPs, such as marinades and coatings, were entered following the practices described above.

Once all foods had been entered for each participant, the records were submitted electronically and checked for potential errors by Erin Dwyer (Research dietitian, Monash University). A report was then generated in Microsoft Excel containing total and individual fermentable carbohydrate intakes for each participant, which was used for further analysis.

### 2.4.8.5 Nutrient intake analysis

Once data had been checked for errors in data entry (section 2.4.8.3), the four study groups were compared for absolute intakes of all major macronutrients, micronutrients, fibre and FODMAPs.

Nutritional adequacy was explored by investigating the proportion of participants achieving nutrient recommendations. For micronutrients, this was defined as meeting or exceeding recommendations outlined by the Scientific Advisory Committee on Nutrition (SACN, 2016, DoH, 1991, PHE, 2016). Calcium requirements are greater in IBD (1000 mg/day) (Scott et al., 2000) than in the general adult population (700 mg/day), therefore these requirements were used for patients with IBD, however to enable consistent cut-offs for achievement of the RNI calcium was also compared against 700 mg/d for all participants. Macronutrient recommendations are expressed as either the minimum (e.g. carbohydrate) or maximum (e.g. fat) proportion of energy they should contribute to the diet (SACN, 2015).
2.4.9 Questionnaires

2.4.9.1 IBD control

The concept and measurement of IBD control will be discussed in Chapter 4 (section 4.4.3). The validated IBD-control questionnaire was completed by patients in this study in order to correlate dietary intake and FR-QOL with patient-perceived control of IBD (Bodger et al., 2013).

2.4.9.2 Food-related quality of life

Inflammatory bowel disease can have an impact not only upon nutritional adequacy but also on the psychosocial aspects of food and mealtimes. Food-related quality of life (FR-QOL) encompasses the pleasure derived from food and the social activities involving eating and drinking, as well as the ability to achieve nutritional adequacy (Gustafsson and Draper, 2009, Whit, 2001). Food-related quality of life can be impacted by the GI symptoms characteristic of IBD and food restrictions imposed by many patients. A validated questionnaire designed to assess issues relating to food and nutrition in IBD has shown that FR-QOL is significantly poorer in IBD than in asthma and healthy controls (Hughes et al., 2016). This validated questionnaire will be used to assess FR-QOL in the current trial and is based upon 29 questions with responses on a 5-point Likert scale from 'strongly agree' to 'strongly disagree', pertaining to the impact of IBD on enjoyment of mealtimes, social activities involving food and the ability to maintain adequate nutrition.

For the healthy controls, the wording of the questionnaire was adapted, for example, instead of "I have regretted eating and drinking things that have upset my IBD", the wording in the healthy control version is "I have regretted eating and drinking things that have upset my digestion".

2.4.10 Sample size calculation

There is a paucity of data regarding fermentable carbohydrate intakes in patients with IBD. A previous case-control study from our group established a significant difference in fructan intakes between patients with active IBD and healthy controls (Anderson et al., 2015). However, due to the small differences in fructan intakes relative to the standard deviations observed between the two groups (active IBD 3.3 SD 2.2 g/day vs. healthy controls 4.2 SD 2.0 g/day), a power-based sample size calculation would result in unfeasible numbers of
participants required to provide sufficient power to detect a difference in fructan intakes across the groups.

For this reason, a precision-based sample size estimation was performed. It was determined that a sample size of 80 participants per group (400 total) would allow an estimation of the mean fructan intakes in the different groups with a reasonable precision (±0.074 g/d of the population mean) (Janet and Philip). Therefore, the aim was to obtain food diaries from 80 patients per group (active IBD, inactive IBD with FGS, inactive IBD without FGS and healthy controls).

2.4.11 Statistical analysis

Data analysis was performed by the thesis author using IBM SPSS Statistics for Windows Version 24.0, after the last participant’s data was collected. Prior to statistical analysis, continuous data were explored for normality via visual inspection of histograms. An analysis of variance (ANOVA) was used to compare normally-distributed continuous variables and a Kruskal-Wallis test was used to compare non-normally distributed continuous variables across the four groups, with Bonferroni correction for pairwise comparisons between two groups. A Chi-squared test was used to compare categorical variables across groups. Pearson or Spearman correlations were used to investigate correlations between outcomes and multiple linear regression analysis was used to investigate factors (e.g. age, gender, IBD diagnosis and disease activity) associated with fermentable carbohydrate intake.

2.5 Results: FODMAP and nutrient intakes in patients with active and inactive IBD compared to healthy controls

2.5.1 Patient recruitment and progress

Recruitment, data collection, data entry and statistical analysis was conducted by the author of this thesis, except for a subset of 10 healthy controls who were recruited with the assistance of Alice Wright (student dietitian, King’s College London).

Figure 2.1 displays the numbers of patients that were screened, consented and returned food diaries, per study group. The target sample size of 80 participants per group was not obtained in time to include in this thesis, and therefore data for participants recruited to date was analysed and the findings are presented in this section.
Chapter 2: Case-control study of dietary intake in IBD

Figure 2.1 Participant flow through the case-control study. Diaries for participants with inactive IBD and FGS were the baseline food diaries collected as part of a randomised controlled trial of the low FODMAP diet in patients with inactive IBD (Chapters 4-6 of this thesis).

Upon initial screening, four patients with active IBD were excluded; two with a stoma, one with current stricturing CD and one with a significant comorbidity. Of the patients with inactive IBD with FGS screened (n=155), the major reasons for exclusion were declining to participate or loss during screening, elevated inflammatory markers, taking antibiotics or probiotics, or GI symptoms not meeting the specified criteria (for further details see section 5.2.1.3 and 5.2.1). Six patients with inactive IBD without FGS were excluded; three with a high serum CRP, one with a significant comorbidity, one currently following a low FODMAP diet and one due to pregnancy. The numbers of patients screened and excluded in this group are higher than the other study groups because these patients were recruited as part of a RCT with additional inclusion and exclusion criteria. One healthy control was excluded due to fulfilling the criteria for irritable bowel syndrome.

Diaries and questionnaires were returned by twenty-one patients in the active IBD group (34% of those consented), thirty-eight in the inactive IBD without FGS group (35%), fifty-two in the inactive IBD with FGS group (100% of consented; these were collected as part of the RCT described in Chapters 4-6) and thirty-six (68%) in the healthy control group. These diaries and questionnaires were analysed and the findings are presented in this section.
2.5.2 Demographic and disease characteristics of participants

The demographic characteristics of the participants in the four study groups are presented in Table 2.2. The proportion of males and females was different across the groups (P=0.004). Pairwise comparisons revealed more females among patients with inactive IBD without FGS (26/38, 68%) and healthy controls (27/36, 75%) than patients with active IBD (6/21, 29%). There was a difference in the maximum educational qualification between the groups with higher numbers with advanced qualifications in the healthy controls (Table 2.2).

### Table 2.2 Demographic characteristics participants in the four study groups

<table>
<thead>
<tr>
<th></th>
<th>Active IBD (n=21)</th>
<th>Inactive IBD with FGS (n=52)</th>
<th>Inactive IBD without FGS (n=38)</th>
<th>Healthy controls (n=36)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), median (IQR)</td>
<td>40 (21)</td>
<td>35 (18)</td>
<td>34 (18)</td>
<td>32 (21)</td>
<td>0.483</td>
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<td>29 (56)a,b</td>
<td>26 (68)b</td>
<td>27 (75)b</td>
<td>0.004</td>
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<td></td>
<td></td>
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<td>25 (66)</td>
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<td></td>
</tr>
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<td>14 (27)</td>
<td>8 (21)</td>
<td>6 (17)</td>
<td></td>
</tr>
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<td>0 (0)</td>
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<td></td>
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<tr>
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<td>0 (0)</td>
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<td></td>
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<td>2 (4)</td>
<td>1 (3)</td>
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<td>0 (0)</td>
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</tr>
<tr>
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<td>0 (0)</td>
<td>2 (6)</td>
<td></td>
</tr>
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<td>Caribbean</td>
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<td>1 (2)</td>
<td>1 (3)</td>
<td>2 (6)</td>
<td></td>
</tr>
<tr>
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<td>0 (0)</td>
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</tr>
<tr>
<td>Any other ethnic group</td>
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<td>0 (0)</td>
<td>1 (3)</td>
<td>1 (3)</td>
<td></td>
</tr>
<tr>
<td>Arab</td>
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<td>0 (0)</td>
<td>1 (3)</td>
<td></td>
</tr>
<tr>
<td>Maximum education level</td>
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<td></td>
<td></td>
<td>0.017</td>
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<tr>
<td>No formal qualifications</td>
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<td>0 (0)</td>
<td>1 (3)</td>
<td>1 (3)</td>
<td></td>
</tr>
<tr>
<td>Vocational</td>
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<td>3 (6)</td>
<td>0 (0)</td>
<td>1 (3)</td>
<td></td>
</tr>
<tr>
<td>School-level (e.g. GCSE)</td>
<td>3 (14)a,b</td>
<td>14 (27)a</td>
<td>9 (24)a</td>
<td>0 (0)b</td>
<td></td>
</tr>
<tr>
<td>Advanced (e.g. A-level)</td>
<td>6 (29)</td>
<td>9 (17)</td>
<td>3 (8)</td>
<td>7 (19)</td>
<td></td>
</tr>
<tr>
<td>University degree</td>
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<td>23 (44)</td>
<td>17 (45)</td>
<td>13 (36)</td>
<td></td>
</tr>
<tr>
<td>Postgraduate degree</td>
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<td>3 (6)a</td>
<td>6 (16)a,b</td>
<td>12 (33)b</td>
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<tr>
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<td>Smoking status</td>
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<td></td>
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<td>Current smoker</td>
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<td>1 (3)</td>
<td>1 (3)</td>
<td></td>
</tr>
<tr>
<td>Previous smoker</td>
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<td>13 (25)</td>
<td>7 (18)</td>
<td>3 (8)</td>
<td></td>
</tr>
<tr>
<td>Non-smoker</td>
<td>13 (62)</td>
<td>34 (65)</td>
<td>30 (79)</td>
<td>32 (89)</td>
<td></td>
</tr>
<tr>
<td>Body weight (kg), median (IQR)</td>
<td>71 (16)a,b</td>
<td>70 (21)a</td>
<td>68 (17)a,b</td>
<td>62 (15)b</td>
<td>0.039</td>
</tr>
<tr>
<td>Height (m), median (IQR)</td>
<td>1.8 (0.2)a,b</td>
<td>1.7 (0.2)a,b</td>
<td>1.7 (0.1)a</td>
<td>1.7 (0.1)b</td>
<td>0.022</td>
</tr>
<tr>
<td>BMI (kg/m²), median (IQR)</td>
<td>23 (4)a,b</td>
<td>24 (6)a</td>
<td>23 (4)a,b</td>
<td>22 (4)b</td>
<td>0.028</td>
</tr>
</tbody>
</table>

Values are number of participants (% of group), unless otherwise stated. Groups were compared using Chi-squared test except for continuous variables, compared across groups using Kruskal-Wallis test. Columns without superscript letters in common are significantly different at the 0.05 level.
Body weight was different across the study groups (P=0.039), with greater body weight in inactive IBD with FGS compared to healthy controls. In line with this, body mass index (BMI) was different across the groups (P=0.028), with greater BMI in patients with inactive IBD with FGS compared to healthy controls.

Table 2.3 displays the disease characteristics of patients with IBD. Across the IBD groups, there was a difference in the proportion of patients with CD (P=0.029), with fewer CD in the active IBD group (7/21, 33%) than the inactive IBD without FGS group (26/38, 68%). Of the patients with inactive IBD with FGS, 29% met criteria for diarrhoea-predominant irritable bowel syndrome (IBS), 8% mixed IBS, 2% unsubtyped IBS, 54% functional bloating and 8% functional diarrhoea. As expected, significantly more patients in the active IBD group were currently taking steroids, compared to patients with inactive IBD (P<0.001 across groups).
### Table 2.3 Disease characteristics of patients with IBD

<table>
<thead>
<tr>
<th></th>
<th>Active IBD (n=21)</th>
<th>Inactive IBD with FGS (n=52)</th>
<th>Inactive IBD without FGS (n=38)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
<td><strong>0.029</strong></td>
</tr>
<tr>
<td>Crohn’s disease</td>
<td>7/21 (33)</td>
<td>26/52 (50)</td>
<td>26/38 (68)</td>
<td></td>
</tr>
<tr>
<td>Crohn’s disease location</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ileal</td>
<td>1/7 (14)</td>
<td>6/26 (12)</td>
<td>5/26 (19)</td>
<td>0.917</td>
</tr>
<tr>
<td>Colonic</td>
<td>3/7 (43)</td>
<td>8/26 (15)</td>
<td>11/26 (42)</td>
<td></td>
</tr>
<tr>
<td>Ileocolonic</td>
<td>3/7 (43)</td>
<td>12/26 (23)</td>
<td>10/26 (38)</td>
<td></td>
</tr>
<tr>
<td>Perianal disease</td>
<td>1/7 (14)</td>
<td>6/26 (12)</td>
<td>6/26 (23)</td>
<td>0.451</td>
</tr>
<tr>
<td><strong>Crohn’s disease behaviour</strong></td>
<td></td>
<td></td>
<td></td>
<td><strong>0.594</strong></td>
</tr>
<tr>
<td>Non-stricturing, non-penetrating</td>
<td>4/7 (19)</td>
<td>17/26 (33)</td>
<td>15/26 (58)</td>
<td></td>
</tr>
<tr>
<td>Stricturing</td>
<td>3/7 (14)</td>
<td>5/26 (10)</td>
<td>6/26 (23)</td>
<td></td>
</tr>
<tr>
<td>Penetrating</td>
<td>0/7 (0)</td>
<td>4/26 (8)</td>
<td>5/26 (19)</td>
<td></td>
</tr>
<tr>
<td><strong>HBI score (CD only), median (IQR)</strong></td>
<td></td>
<td></td>
<td></td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td>Crohn’s disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ileal</td>
<td>8 (4)</td>
<td>4 (2)</td>
<td>0 (1)</td>
<td></td>
</tr>
<tr>
<td>Colonic</td>
<td>4/7 (57)</td>
<td>5/26 (19)</td>
<td>11/26 (42)</td>
<td>0.062</td>
</tr>
<tr>
<td>Ileocolonic</td>
<td>3/7 (43)</td>
<td>12/26 (23)</td>
<td>10/26 (38)</td>
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</tr>
<tr>
<td>Perianal disease</td>
<td>1/7 (14)</td>
<td>6/26 (12)</td>
<td>6/26 (23)</td>
<td>0.451</td>
</tr>
<tr>
<td>Mediation</td>
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<td>15 (29)</td>
<td>21 (55)</td>
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<tr>
<td>Steroids</td>
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<td>0 (0)</td>
<td>0 (0)</td>
<td><strong>&lt;0.001</strong></td>
</tr>
</tbody>
</table>

**All values are number of participants (percent of group) unless otherwise stated. Groups were compared using a Chi-squared test or Kruskal-Wallis test. Columns without superscript letters in common are significantly different at the 0.05 level.**
2.5.3 Under-reporting and over-reporting

There was no difference across the study groups in the proportion of participants classified as under-reporting, acceptable-reporting or over-reporting of energy intake (P=0.756) (Table 2.4). For the participants classified as acceptable energy reporters, an average of 2% of energy intake was under-reported, while in under-reporters an average of 34% energy intake was under-reported, and in over-reporters an average of 34% energy intake was over-reported.

Table 2.4 Proportion of study groups classified as under-reporters, acceptable reporters and over-reporters of energy

<table>
<thead>
<tr>
<th></th>
<th>Active IBD (n=21)</th>
<th>Inactive IBD with FGS (n=52)</th>
<th>Inactive IBD without FGS (n=38)</th>
<th>Healthy controls (n=36)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Under-reporters</td>
<td>7 (33)</td>
<td>15 (29)</td>
<td>6 (16)</td>
<td>8 (22)</td>
<td>0.756</td>
</tr>
<tr>
<td>Acceptable</td>
<td>11 (52)</td>
<td>31 (60)</td>
<td>27 (71)</td>
<td>24 (67)</td>
<td></td>
</tr>
<tr>
<td>Over-reporters</td>
<td>3 (14)</td>
<td>6 (12)</td>
<td>5 (13)</td>
<td>4 (11)</td>
<td></td>
</tr>
</tbody>
</table>

Groups were compared using a Chi-squared test.

2.5.4 FODMAP intake

All food diaries were analysed for individual and total FODMAP intakes for the four study groups are presented in Table 2.5. Across the four study groups, there were significant differences in GOS (P=0.023), total polyol (P=0.005) and sorbitol (P=0.001) intakes. Pairwise comparisons revealed significantly lower GOS intakes in patients with inactive IBD with FGS compared to healthy controls (0.6 IQR 0.9 g/day vs. 1.0 IQR 1.4 g/day, P=0.019). In terms of total polyol intakes, compared to healthy controls (1.3 IQR 1.4 g/day), intakes were significantly lower in inactive IBD with FGS (0.6 IQR 1.0 g/day, P=0.010) and inactive IBD without FGS (0.8 IQR 0.9 g/day, P=0.015). Compared to sorbitol intakes in healthy controls (0.7 IQR 1.2), intakes were significantly lower in patients with active IBD (0.3 IQR 0.4, P=0.008), inactive IBD with FGS (0.3 IQR 0.7, P=0.009) and inactive IBD without FGS (0.2 IQR 0.7, P=0.001). The difference across the groups in total FODMAP intake expressed per 1000 kcal of energy intake was approaching significance (P=0.056), with a lower intake in patients with active IBD than in the other three groups.

Across the groups, there was no significant difference in the intake of excess fructose, lactose, mannitol or fructans per 1000 kcal of energy intake. Reflecting the findings of the total fermentable carbohydrate intakes, there was significantly lower GOS intake per 1000 kcal of energy intake.
kcal in inactive IBD with FGS compared to healthy controls (P=0.009). There was also significantly lower total polyol intake per 1000 kcal in inactive IBD with FGS (P=0.013) and inactive IBD without FGS compared to healthy controls (P=0.014). Compared to sorbitol intakes per 1000 kcal in healthy controls, intakes were significantly lower in active IBD (P=0.007), inactive IBD with FGS (P=0.011) and inactive IBD without FGS (P=0.001).

Table 2.5 Total and individual FODMAP intakes across the four study groups

<table>
<thead>
<tr>
<th></th>
<th>Active IBD (n=21)</th>
<th>Inactive IBD with FGS (n=52)</th>
<th>Inactive IBD without FGS (n=38)</th>
<th>Healthy controls (n=36)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intakes, g/d</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total FODMAPs</td>
<td>9.0 (14.7)</td>
<td>12.5 (10.0)</td>
<td>15.5 (9.5)</td>
<td>16.8 (8.1)</td>
<td>0.068</td>
</tr>
<tr>
<td>Fructans</td>
<td>2.6 (1.9)</td>
<td>2.8 (1.9)</td>
<td>2.8 (1.2)</td>
<td>3.0 (1.6)</td>
<td>0.749</td>
</tr>
<tr>
<td>Galacto-oligosaccharides</td>
<td>0.9 (1.1)²,³</td>
<td>0.6 (0.9)²</td>
<td>0.8 (0.9)²</td>
<td>1.0 (1.4)²</td>
<td>0.023</td>
</tr>
<tr>
<td>Lactose</td>
<td>3.1 (15.0)</td>
<td>4.7 (9.5)</td>
<td>7.7 (10.1)</td>
<td>7.9 (12.8)</td>
<td>0.344</td>
</tr>
<tr>
<td>Excess fructose</td>
<td>1.1 (1.5)</td>
<td>1.0 (1.1)</td>
<td>1.2 (1.5)</td>
<td>1.6 (2.1)</td>
<td>0.207</td>
</tr>
<tr>
<td>Total polyols</td>
<td>0.6 (0.9)²,³</td>
<td>0.6 (1.0)²</td>
<td>0.8 (0.9)²</td>
<td>1.3 (1.4)²</td>
<td>0.005</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>0.3 (0.4)²</td>
<td>0.3 (0.7)²</td>
<td>0.2 (0.7)²</td>
<td>0.7 (1.2)²</td>
<td>0.001</td>
</tr>
<tr>
<td>Mannitol</td>
<td>0.3 (0.6)</td>
<td>0.2 (0.4)</td>
<td>0.2 (0.6)</td>
<td>0.3 (0.7)</td>
<td>0.435</td>
</tr>
<tr>
<td><strong>Intakes, g/1000 kcal/d</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total FODMAPs</td>
<td>5.3 (6.9)</td>
<td>7.0 (6.7)</td>
<td>7.8 (5.6)</td>
<td>8.6 (4.5)</td>
<td>0.056</td>
</tr>
<tr>
<td>Fructans</td>
<td>1.4 (0.7)</td>
<td>1.6 (0.5)</td>
<td>1.5 (0.5)</td>
<td>1.6 (0.7)</td>
<td>0.144</td>
</tr>
<tr>
<td>Galacto-oligosaccharides</td>
<td>0.5 (0.6)²,³</td>
<td>0.3 (0.4)²</td>
<td>0.4 (0.4)²</td>
<td>0.5 (0.7)²</td>
<td>0.009</td>
</tr>
<tr>
<td>Lactose</td>
<td>2.3 (7.4)</td>
<td>2.4 (6.0)</td>
<td>3.8 (6.4)</td>
<td>4.4 (6.4)</td>
<td>0.288</td>
</tr>
<tr>
<td>Excess fructose</td>
<td>0.5 (0.7)</td>
<td>0.5 (0.6)</td>
<td>0.6 (0.9)</td>
<td>0.8 (0.9)</td>
<td>0.163</td>
</tr>
<tr>
<td>Total polyols</td>
<td>0.4 (0.5)²,³</td>
<td>0.3 (0.5)²</td>
<td>0.4 (0.5)²</td>
<td>0.6 (1.0)²</td>
<td>0.014</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>0.2 (0.2)²</td>
<td>0.2 (0.4)²</td>
<td>0.1 (0.3)²</td>
<td>0.4 (0.5)²</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mannitol</td>
<td>0.2 (0.4)</td>
<td>0.1 (0.2)</td>
<td>0.1 (0.3)</td>
<td>0.1 (0.4)</td>
<td>0.544</td>
</tr>
</tbody>
</table>

All values are median (interquartile range). Groups were compared using Kruskal-Wallis test with pairwise comparisons and Bonferroni correction. Columns without superscript letters in common are significantly different at the 0.05 level. †Fructose in excess of a 1:1 fructose to glucose ratio

There were no differences in fructan intakes between the groups, either in g/d or in g/1000 kcal/d. A Pearson correlation showed total energy and fructan intakes to be strongly positively correlated ($r=0.609$, $P<0.001$). To investigate factors that may predict fructan and total FODMAP intakes multiple regression was conducted. First, two separate multiple
regression analyses including all participants were performed with daily fructan intake and total FODMAP intake as dependent variables. The independent variables for each regression analysis were age, gender, a diagnosis of IBD and the presence of FGS. The multiple regression did not significantly predict fructan intakes \( F(4, 142) = 1.99, P=0.099 \) or total FODMAP intakes \( F(4, 139) = 1.30, P=0.274 \). Next, a multiple regression analysis was conducted in patients with IBD only to predict any IBD characteristics or non-IBD characteristics predicting fructan and total FODMAP intakes in patients. Again, two separate multiple regression analyses were conducted with fructan intake and total FODMAP intake as the dependent variables, while in both analyses the independent variables were gender, diagnosis (CD or UC), the presence of active IBD, the presence of colonic disease (including UC, ileocolonic CD and colonic CD), the presence of FGS and the total IBD control score. The model significantly predicted fructan intakes \( F(5, 105) = 2.63, P=0.028 \). The overall model had an adjusted \( R^2 \) value of 0.069, suggesting it to explain 6.9% of the variation in fructan intakes in IBD. The independent variables that significantly predicted fructan intake were the presence of colonic disease (regression coefficient, \( B \), -0.99, standard error, 0.38, \( P=0.010 \)) and gender (regression coefficient, \( B \), -0.60, standard error, 0.24, \( P=0.015 \)).

### 2.5.5 Nutrient intake

Daily energy, macronutrient and fibre intakes are displayed in Table 2.6. Across the groups, there was a difference in non-starch polysaccharide (NSP) (\( P=0.014 \)) and total fibre (AOAC method) intake (\( P=0.032 \)). Pairwise comparisons revealed significantly lower NSP intake in inactive IBD with FGS compared to healthy controls (12 g/d IQR 11 vs. 17 g/d IQR 10, \( P=0.018 \)) and in inactive IBD without FGS compared to healthy controls (15 g/d IQR 6 vs. 17 g/d IQR 10, \( P=0.037 \)), which also corresponded to different intakes of NSP/1000 kcal/d. Pairwise comparisons revealed significantly lower total fibre (AOAC) intake in inactive IBD with FGS compared to healthy controls (17 IQR 16 g/d vs. 22 IQR 15 g/d, \( P=0.025 \)). Across groups there were no differences in the proportion of energy derived from different macronutrients, except for starch (\( P=0.011 \)), which contributed a greater proportion of energy in active IBD than healthy controls (26 IQR 8% vs. 21 IQR 8%, \( P=0.033 \)).

Daily micronutrient intakes are displayed in Table 2.7. There were differences across groups in potassium (\( P=0.021 \)), copper (\( P=0.006 \)), manganese (\( P=0.006 \)), biotin (vitamin B\(_7\)) (\( P=0.024 \)) and vitamin C (\( P=0.012 \)) intakes. Following pairwise comparison all differences were driven by lower intakes in inactive IBD with FGS compared to healthy controls. For example, there was significantly lower intake of potassium (3059.0 mg/d IQR 1444.7 vs.
3482.0 mg/d IQR 2040.3, P=0.032), copper (1.5 mg/d IQR 0.9 vs. 1.9 mg/d IQR 2.0, P=0.006), manganese (3.2 mg/d IQR 2.6 vs. 4.3 mg/d IQR 4.4, 0.004), biotin (36.2 μg/d IQR 19.0 vs. 46.5 μg/d IQR 20.7, P=0.017) and vitamin C (84.5 mg/d IQR 75.6 vs. 137.0 mg/d IQR 55.1, P=0.010).
### Table 2.6 Daily macronutrient and fibre intakes across the four study groups

<table>
<thead>
<tr>
<th></th>
<th>Active IBD (n=21)</th>
<th>Inactive IBD with FGS (n=52)</th>
<th>Inactive IBD without FGS (n=38)</th>
<th>Healthy controls (n=36)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>2260 (949)</td>
<td>1961 (838)</td>
<td>2131 (629)</td>
<td>1876 (603)</td>
<td>0.504</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>77 (36)</td>
<td>78 (55)</td>
<td>85 (23)</td>
<td>76 (37)</td>
<td>0.883</td>
</tr>
<tr>
<td>Protein (% of total E)</td>
<td>15 (4)</td>
<td>17 (5)</td>
<td>16 (6)</td>
<td>16 (5)</td>
<td>0.832</td>
</tr>
<tr>
<td>Total fat (g)</td>
<td>83 (39)</td>
<td>77 (44)</td>
<td>81 (32)</td>
<td>84 (37)</td>
<td>0.261</td>
</tr>
<tr>
<td>Fat (% of total E)</td>
<td>38 (12)</td>
<td>37 (5)</td>
<td>35 (5)</td>
<td>38 (6)</td>
<td>0.370</td>
</tr>
<tr>
<td>Saturated fat (g)</td>
<td>29 (8)</td>
<td>26 (15)</td>
<td>26 (12)</td>
<td>26 (15)</td>
<td>0.440</td>
</tr>
<tr>
<td>Saturated fat (% of total E)</td>
<td>11 (5)</td>
<td>12 (4)</td>
<td>12 (4)</td>
<td>13 (3)</td>
<td>0.653</td>
</tr>
<tr>
<td>Monounsaturated fat (g)</td>
<td>31 (14)</td>
<td>28 (18)</td>
<td>30 (12)</td>
<td>32 (14)</td>
<td>0.578</td>
</tr>
<tr>
<td>Monounsaturated fat (% of total E)</td>
<td>14 (4)</td>
<td>13 (3)</td>
<td>13 (2)</td>
<td>14 (3)</td>
<td>0.388</td>
</tr>
<tr>
<td>Polyunsaturated fat (%)</td>
<td>14 (6)</td>
<td>13 (7)</td>
<td>13 (8)</td>
<td>13 (7)</td>
<td>0.514</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>234 (138)</td>
<td>199 (117)</td>
<td>236 (85)</td>
<td>172 (98)</td>
<td>0.478</td>
</tr>
<tr>
<td>Carbohydrate (% of total E)</td>
<td>43 (7)</td>
<td>40 (7)</td>
<td>42 (8)</td>
<td>38 (16)</td>
<td>0.666</td>
</tr>
<tr>
<td>Starch (g)</td>
<td>135 (97)</td>
<td>111 (69)</td>
<td>125 (39)</td>
<td>101 (50)</td>
<td>0.085</td>
</tr>
<tr>
<td>Sugars (g)</td>
<td>94 (46)</td>
<td>73 (54)</td>
<td>84 (52)</td>
<td>86 (57)</td>
<td>0.533</td>
</tr>
<tr>
<td>NSP (g)</td>
<td>12 (11)</td>
<td>12 (11)</td>
<td>15 (6)</td>
<td>17 (10)</td>
<td>0.014</td>
</tr>
<tr>
<td>Total fibre (AOAC) (g)</td>
<td>18 (13)</td>
<td>16 (16)</td>
<td>19 (10)</td>
<td>22 (15)</td>
<td>0.032</td>
</tr>
</tbody>
</table>

Values are median (interquartile range). Groups were compared using Kruskal-Wallis test with pairwise comparisons and Bonferroni correction for multiple comparisons. Columns without superscript letters in common are significantly different at the 0.05 level. FGS, functional gastrointestinal symptoms; NSP, non-starch polysaccharide; AOAC, Association of Analytical Chemists
Table 2.7 Micronutrient intakes across the four study groups

<table>
<thead>
<tr>
<th>Nutrient (mg/μg)</th>
<th>Active IBD (n=21)</th>
<th>Inactive IBD with FGS (n=52)</th>
<th>Inactive IBD without FGS (n=38)</th>
<th>Healthy controls (n=36)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (mg)</td>
<td>2194.7 (1080.5)</td>
<td>2186.9 (1048.5)</td>
<td>2424.7 (665.8)</td>
<td>2040.6 (925.4)</td>
<td>0.285</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>2912.8 (1055.8)</td>
<td>3059.0 (1444.7)</td>
<td>3077.0 (968.2)</td>
<td>3482.0 (2040.3)</td>
<td>0.048</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>947.7 (562.9)</td>
<td>801.4 (638.4)</td>
<td>947.3 (379.3)</td>
<td>858.9 (279.1)</td>
<td>0.433</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>324.2 (168.1)</td>
<td>297.2 (219.1)</td>
<td>328.4 (103.6)</td>
<td>332.8 (168.2)</td>
<td>0.055</td>
</tr>
<tr>
<td>Phosphorous (mg)</td>
<td>1369.1 (791.3)</td>
<td>1219.9 (846.7)</td>
<td>1343.1 (378.3)</td>
<td>1315.1 (578.2)</td>
<td>0.401</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>11.2 (5.8)</td>
<td>11.0 (7.1)</td>
<td>11.1 (3.4)</td>
<td>13.0 (7.3)</td>
<td>0.095</td>
</tr>
<tr>
<td>Copper (mg)</td>
<td>1.5 (1.2)</td>
<td>1.5 (0.9)</td>
<td>1.4 (0.5)</td>
<td>1.9 (2.0)</td>
<td>0.006</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>8.1 (6.1)</td>
<td>8.5 (6.0)</td>
<td>9.0 (2.4)</td>
<td>9.6 (11.4)</td>
<td>0.447</td>
</tr>
<tr>
<td>Chloride (mg)</td>
<td>3531.0 (1839.3)</td>
<td>3297.3 (1695.2)</td>
<td>3310.4 (1310.2)</td>
<td>3140.5 (1247.0)</td>
<td>0.415</td>
</tr>
<tr>
<td>Manganese (mg)</td>
<td>3.9 (3.5)</td>
<td>3.2 (2.6)</td>
<td>3.6 (1.7)</td>
<td>4.3 (4.4)</td>
<td>0.006</td>
</tr>
<tr>
<td>Selenium (μg)</td>
<td>48.7 (19.9)</td>
<td>52.5 (27.7)</td>
<td>56.6 (21.5)</td>
<td>61.6 (31.7)</td>
<td>0.557</td>
</tr>
<tr>
<td>Iodine (μg)</td>
<td>85.2 (119.4)</td>
<td>115.8 (85.5)</td>
<td>134.5 (65.5)</td>
<td>114.9 (54.2)</td>
<td>0.493</td>
</tr>
<tr>
<td>Vitamin A (μg)</td>
<td>869.8 (621.4)</td>
<td>1085.8 (1969.1)</td>
<td>721.4 (604.4)</td>
<td>1167.5 (609.6)</td>
<td>0.647</td>
</tr>
<tr>
<td>Vitamin D (μg)</td>
<td>3.8 (2.2)</td>
<td>3.3 (3.0)</td>
<td>3.0 (1.3)</td>
<td>2.3 (3.1)</td>
<td>0.131</td>
</tr>
<tr>
<td>Vitamin E (mg)</td>
<td>10.1 (7.3)</td>
<td>11.8 (7.3)</td>
<td>9.5 (5.4)</td>
<td>11.4 (6.5)</td>
<td>0.121</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>78.9 (92.8)</td>
<td>84.5 (75.6)</td>
<td>116.5 (66.8)</td>
<td>137.0 (55.1)</td>
<td>0.012</td>
</tr>
<tr>
<td>Vitamin K1 (μg)</td>
<td>56.0 (90.6)</td>
<td>53.4 (80.8)</td>
<td>69.4 (83.9)</td>
<td>102.1 (98.7)</td>
<td>0.394</td>
</tr>
<tr>
<td>Thiamin (vitamin B1) (mg)</td>
<td>1.7 (0.7)</td>
<td>1.5 (0.7)</td>
<td>1.4 (0.7)</td>
<td>1.6 (0.9)</td>
<td>0.500</td>
</tr>
<tr>
<td>Riboflavin (vitamin B2) (mg)</td>
<td>1.5 (0.8)</td>
<td>1.6 (1.1)</td>
<td>1.6 (0.8)</td>
<td>1.7 (0.7)</td>
<td>0.265</td>
</tr>
<tr>
<td>Niacin (vitamin B3) (mg)</td>
<td>33.8 (19.8)</td>
<td>39.8 (26.1)</td>
<td>36.5 (9.4)</td>
<td>35.2 (17.3)</td>
<td>0.568</td>
</tr>
</tbody>
</table>
### Chapter 2: Case-control study of dietary intake in IBD

<table>
<thead>
<tr>
<th></th>
<th>Active IBD (n=21)</th>
<th>Inactive IBD with FGS (n=52)</th>
<th>Inactive IBD without FGS (n=38)</th>
<th>Healthy controls (n=36)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pantothenate (vitamin B₅) (mg)</td>
<td>5.1 (2.5)</td>
<td>6.0 (2.5)</td>
<td>5.7 (1.7)</td>
<td>6.0 (3.4)</td>
<td>0.908</td>
</tr>
<tr>
<td>Pyridoxine (vitamin B₆) (mg)</td>
<td>2.3 (1.2)</td>
<td>2.0 (1.1)</td>
<td>1.9 (0.5)</td>
<td>1.8 (0.8)</td>
<td>0.416</td>
</tr>
<tr>
<td>Biotin (vitamin B₇) (μg)</td>
<td>36.9 (29.5)ᵃᵇ</td>
<td>36.2 (19.0)ᵃ</td>
<td>36.3 (13.6)ᵃᵇ</td>
<td>46.5 (20.7)ᵇ</td>
<td>0.024</td>
</tr>
<tr>
<td>Folate (μg)</td>
<td>268.4 (162.9)</td>
<td>203.4 (121.1)</td>
<td>202.7 (141.8)</td>
<td>266.6 (79.4)</td>
<td>0.079</td>
</tr>
<tr>
<td>Cobalamin (vitamin B₁₂) (μg)</td>
<td>5.5 (2.1)</td>
<td>5.3 (3.2)</td>
<td>4.4 (2.8)</td>
<td>3.7 (4.2)</td>
<td>0.416</td>
</tr>
</tbody>
</table>

Values are median (interquartile range). Groups were compared using Kruskal-Wallis test with pairwise comparisons and Bonferroni correction for multiple comparisons. Columns without superscript letters in common are significantly different at the 0.05 level. FGS, functional gastrointestinal symptoms.
2.5.6 Nutritional adequacy

Table 2.8 shows the proportions of participants in the study groups meeting recommendations for macronutrient and micronutrient intakes. Across groups, there was a significant difference in the proportion of participants meeting recommendations for fibre (AOAC) \((P=0.047)\), calcium \((P<0.001)\), riboflavin (vitamin B\(_2\)) \((P=0.025)\) and folate \((P=0.018)\). Upon pairwise comparisons, no differences in the proportion of participants meeting fibre or riboflavin recommendations were observed between any of the study groups. The proportion of participants meeting calcium recommendations was lower in all the IBD groups compared to the healthy control group, while the proportion meeting the folate recommendation was lower in patients with inactive IBD with FGS compared to healthy controls. When a calcium requirement of 700 mg/d was assumed for all study groups, there was no difference across the study groups in the proportion of patients meeting the requirement. This suggests that the difference across groups was driven by different requirements assumed for IBD groups (1000 mg/d) and healthy controls (700 mg/d).
Table 2.8 The proportion of participants achieving recommendations for macronutrients, fibre and micronutrients across the four study groups

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Active IBD (n=21)</th>
<th>Inactive IBD with FGS (n=52)</th>
<th>Inactive IBD without FGS (n=38)</th>
<th>Healthy controls (n=36)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (0.75 g/kg weight)</td>
<td>20 (95)</td>
<td>47 (90)</td>
<td>37 (97)</td>
<td>34 (94)</td>
<td>0.573</td>
</tr>
<tr>
<td>Fat (≤30% energy)</td>
<td>6 (29)</td>
<td>18 (35)</td>
<td>17 (45)</td>
<td>13 (36)</td>
<td>0.625</td>
</tr>
<tr>
<td>Saturated fat (≤11% energy)</td>
<td>7 (33)</td>
<td>15 (29)</td>
<td>15 (40)</td>
<td>13 (36)</td>
<td>0.753</td>
</tr>
<tr>
<td>Carbohydrate (≥50% energy)</td>
<td>2 (10)</td>
<td>9 (17)</td>
<td>7 (18)</td>
<td>5 (14)</td>
<td>0.799</td>
</tr>
<tr>
<td>Free sugars (≤5% energy)</td>
<td>5 (24)</td>
<td>15 (29)</td>
<td>9 (24)</td>
<td>11 (31)</td>
<td>0.888</td>
</tr>
<tr>
<td>Fibre (AOAC) (30 g/day)</td>
<td>2 (10)</td>
<td>8 (15)</td>
<td>2 (5)</td>
<td>10 (28)</td>
<td><strong>0.047</strong></td>
</tr>
<tr>
<td>Potassium</td>
<td>6 (29)</td>
<td>12 (23)</td>
<td>11 (29)</td>
<td>17 (47)</td>
<td>0.109</td>
</tr>
<tr>
<td>Calcium (all 700 mg/d)</td>
<td>14 (67)</td>
<td>29 (56)</td>
<td>30 (79)</td>
<td>26 (72)</td>
<td>0.117</td>
</tr>
<tr>
<td>Calcium (1000 and 700 mg/d)</td>
<td>4 (19)</td>
<td>12 (23)</td>
<td>13 (34)</td>
<td>26 (72)</td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td>Magnesium</td>
<td>11 (52)</td>
<td>27 (52)</td>
<td>25 (66)</td>
<td>28 (78)</td>
<td>0.069</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>21 (100)</td>
<td>52 (100)</td>
<td>38 (100)</td>
<td>36 (97)</td>
<td>0.376</td>
</tr>
<tr>
<td>Iron</td>
<td>14 (67)</td>
<td>18 (35)</td>
<td>17 (45)</td>
<td>16 (44)</td>
<td>0.101</td>
</tr>
<tr>
<td>Zinc</td>
<td>14 (67)</td>
<td>31 (60)</td>
<td>26 (68)</td>
<td>30 (83)</td>
<td>0.131</td>
</tr>
<tr>
<td>Selenium</td>
<td>5 (24)</td>
<td>12 (23)</td>
<td>13 (34)</td>
<td>16 (44)</td>
<td>0.156</td>
</tr>
<tr>
<td>Iodine</td>
<td>6 (29)</td>
<td>14 (27)</td>
<td>11 (29)</td>
<td>11 (31)</td>
<td>0.986</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>14 (67)</td>
<td>33 (64)</td>
<td>26 (68)</td>
<td>27 (75)</td>
<td>0.724</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>1 (5)</td>
<td>1 (2)</td>
<td>0 (0)</td>
<td>1 (3)</td>
<td>0.643</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>21 (100)</td>
<td>52 (100)</td>
<td>38 (100)</td>
<td>36 (100)</td>
<td>NA</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>18 (86)</td>
<td>41 (79)</td>
<td>33 (87)</td>
<td>33 (92)</td>
<td>0.405</td>
</tr>
<tr>
<td>Vitamin K</td>
<td>7 (33)</td>
<td>17 (33)</td>
<td>17 (45)</td>
<td>19 (53)</td>
<td>0.233</td>
</tr>
</tbody>
</table>
### Chapter 2: Case-control study of dietary intake in IBD

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Active IBD (n=21)</th>
<th>Inactive IBD with FGS (n=52)</th>
<th>Inactive IBD without FGS (n=38)</th>
<th>Healthy controls (n=36)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiamin (vitamin B&lt;sub&gt;1&lt;/sub&gt;)</td>
<td>21 (100)</td>
<td>47 (90)</td>
<td>38 (100)</td>
<td>34 (95)</td>
<td>0.127</td>
</tr>
<tr>
<td>Riboflavin (vitamin B&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>17 (81)</td>
<td>37 (71)</td>
<td>35 (92)</td>
<td>33 (92)</td>
<td><strong>0.025</strong></td>
</tr>
<tr>
<td>Niacin (vitamin B&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>21 (100)</td>
<td>52 (100)</td>
<td>38 (100)</td>
<td>35 (97)</td>
<td>0.376</td>
</tr>
<tr>
<td>Pyridoxine (vitamin B&lt;sub&gt;6&lt;/sub&gt;)</td>
<td>20 (95)</td>
<td>47 (90)</td>
<td>35 (92)</td>
<td>32 (89)</td>
<td>0.863</td>
</tr>
<tr>
<td>Folate</td>
<td>13 (62)&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>30 (42)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26 (68)&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>26 (72)&lt;sup&gt;b&lt;/sup&gt;</td>
<td><strong>0.018</strong></td>
</tr>
<tr>
<td>Cobalamin (vitamin B&lt;sub&gt;12&lt;/sub&gt;)</td>
<td>21 (100)</td>
<td>49 (94)</td>
<td>37 (97)</td>
<td>35 (97)</td>
<td>0.629</td>
</tr>
</tbody>
</table>

All values are number of participants (percent of group). Groups were compared using a Chi-squared test. Columns without superscript letters in common are significantly different at the 0.05 level.
2.5.7 IBD-control

The total IBD-control score was significantly different across the IBD groups (P<0.001). Pairwise comparisons revealed that patients with active IBD (45.5 IQR 50.0) and inactive IBD with FGS (75.5 IQR 35.0) both had significantly lower perceived IBD control than inactive IBD without FGS (116.0 IQR 19.0) (P<0.001 and P<0.001) (Figure 2.2).

Figure 2.2 Box and whisker plot depicting IBD control score in active IBD, inactive IBD with FGS and inactive IBD without FGS (P<0.001 across the groups; P<0.001 active IBD and inactive IBD with FGS vs. inactive IBD without FGS)

2.5.8 Food-related quality of life

There was a significant difference in total FR-QOL score across the four study groups (P<0.001). Pairwise comparisons showed a significantly lower FR-QOL score in patients with active IBD compared to healthy controls (64.0 IQR 24.0 vs. 119.0 IQR 26.0, P<0.001), in patients with inactive IBD with FGS compared to healthy controls (74.0 IQR 26.0 vs. 119.0 IQR 26.0, P<0.001), in patients with active IBD compared to inactive IBD without FGS (65.0 IQR 24.0 vs. 108 IQR 58.0, P<0.001) and in patients with inactive IBD with FGS compared to without FGS (74.0 IQR 26.0 vs. 108.0 IQR 58.0, P<0.001) (Figure 2.3). A Pearson correlation was conducted and showed a strong positive correlation between IBD-control score and FR-QOL score (r=0.670, P<0.001).
Figure 2.3 Box and whisker plot depicting total FR-QOL score in the four study groups (P<0.001 across the groups; P<0.001 for all IBD groups compared to healthy controls)
2.6 Discussion

2.6.1 FODMAP intake

In this case-control study, FODMAP and nutrient intakes and FR-QOL were explored in patients with active IBD (inflammatory GI symptoms), inactive IBD with FGS (functional GI symptoms), inactive IBD without FGS (no GI symptoms), and healthy controls (no GI symptoms). Total fermentable carbohydrate intakes were similar across the study groups, while intakes of GOS, total polyols and sorbitol were significantly lower in IBD compared to healthy controls. When interpreting the findings of this study, it is important to note that the target sample size was not achieved for any of the groups, and the number of patients with active IBD included in the analysis is approximately half that of the other groups. Therefore, when the target sample size is reached, final conclusions regarding accepting or rejecting the hypothesis can be made.

Fructan intake were similar across the four study groups. Fructans are found in foods commonly eaten in the UK, including bread, pasta, onion and garlic, and unsurprisingly fructans therefore make the greatest contribution to total FODMAP intakes in the UK diet (Staudacher et al., 2017b, Staudacher et al., 2012). Fructan intakes were comparable to those observed in a case-control study of patients with active CD (median 2.6 g/day here vs. 2.9 g/day in previous study), inactive CD (median 2.8 g/day here vs. 3.6 g/day in the previous study) and healthy controls (median 3.0 g/day here vs. 3.9 g/day in the previous study) (Anderson et al., 2015). Although there was no statistical difference in fructan intake across groups in the current study, the numerically lowest intake was in active IBD, followed by inactive IBD with and without FGS, followed by healthy controls; the expected pattern according to the previous study (Anderson et al., 2015). However, these values are not statistically significantly different between groups, perhaps a result of a type II error resulting from inadequate power at this stage of recruitment.

The lack of difference between study groups in this study compared to the previous study (Anderson et al., 2015) may relate to the different methods of assessing fructan intakes, with the previous study using a fructan-specific food frequency questionnaire (FFQ) (Dunn et al., 2011), as opposed to 7-day food diaries in the current study, with some food frequency questionnaires over-estimating intakes (Fallaize et al., 2014, Flood et al., 2004, Barclay et al., 2008). On the other hand, the FFQ clearly does not include all the fructan-containing foods that exist and this could result in underestimation of fructan intakes. Furthermore, the current study included patients with UC as well as CD and dietary differences may exist between the conditions. Similar to the other analyses for this study, the small number of participants in the active IBD group compared to the other groups may have limited the power to detect a difference between groups. A possible explanation for similar fructan intakes across groups may
relate to the fact that fructans are derived from a wider range of foods than, say, GOS, which are generally limited to pulses and nuts. Therefore, it may be less likely that fructan restriction will occur unintentionally from exclusion of specific foods. For example, white bread contains fructans and consumption is greater than other cereal products in the UK (Pot et al., 2015, Whelan et al., 2011). Even if a patient were restricting fibre during active IBD or for the management of GI symptoms, white bread would be permitted. On the other hand, fibre-rich (and GOS-containing) pulses and nuts would be limited. Therefore, it is possible that fructan intake is maintained despite dietary restrictions for IBD or GI symptoms.

Regression analysis revealed that lower fructan intakes in patients with IBD were associated with the presence of colonic disease.

There was a lower GOS intake in patients with inactive IBD with FGS compared to healthy controls. Intakes of GOS in the healthy control group in this study were comparable to those observed in the control group of the RCT described in Chapters 4-6. These were in line with the GOS intakes of patients with IBS in the placebo-control group of a recent RCT of the low FODMAP diet (Staudacher et al., 2017b). Meanwhile, GOS intake in inactive IBD with FGS were comparable to those observed in the low FODMAP diet groups of previous trials (Staudacher et al., 2017b, Bohn et al., 2015). This is unlikely to relate to lower energy intake in inactive IBD with FGS because energy intake was not different across the groups and GOS intake remained significantly lower when expressed per 1000 kcal/d. The most likely explanation for lower GOS intake in this group relates to the effect of GOS on GI symptoms. Several cross-sectional studies indicate that pulses and nuts, the major dietary sources of GOS, are perceived symptom triggers in a proportion of patients with IBD (Vagianos et al., 2016, Holt et al., 2017) and also in patients with IBS (Bohn et al., 2013b). A trial of GOS supplementation in IBS showed a better clinical response to a low dose than high dose GOS, and this may be a consequence of increased GI symptoms (such as bloating) following the high dose GOS (Silk et al., 2009). However, the B-GOS (or trans-galactooligosaccharide) used in this trial is distinct from the α-GOS found naturally in pulses and nuts. The lower GOS intake in patients with inactive IBD with FGS may be a result of excluding foods noted to worsen GI symptoms and may represent a long-term dietary modification, but were not the result of following a clinician-advised low FODMAP diet since these patients were known to be naïve to the low FODMAP diet. Galacto-oligosaccharides stimulate a range of GI bacteria, including immune-modulatory bacteria such as F. prausnitzii (Fernando et al., 2010) (Vulevic et al., 2004), and the effects of long-term restriction on immunology and IBD activity are unknown.

Lactose intake was not significantly different across the study groups. This is surprising in view of multiple reports of dairy restriction in IBD (Limdi et al., 2016, Cohen et al., 2013). One study
revealed 37% of patients with IBD perceived milk to worsen GI symptoms (Prince et al., 2011), while another found 12% of patients always avoid milk and milk products (Vagianos et al., 2016). Lactose intakes were lowest in active IBD and highest in the healthy control group, while inactive IBD with FGS had an intermediate intake and inactive IBD without FGS had a similar intake to healthy controls. Given that there is clearly a difference in lactose intake between the active IBD and healthy control group, the lack of a statistically significant difference across groups may again be explained by the small sample size in all groups but particularly in the active IBD group. Lactose intakes in healthy controls in this study were similar to those in the control groups of previous trials of the low FODMAP diet in IBS (Staudacher et al., 2017b, Staudacher et al., 2012, Bohn et al., 2015), and only slightly lower than healthy Swedish individuals (Larsson et al., 2004), suggesting realistic lactose intakes in this study.

Sorbitol intakes were lower in the active IBD, inactive IBD with FGS and inactive IBD without FGS than in healthy controls. Sorbitol is a polyol present in stone fruits and added during manufacturing to sweetened products and sugar-free chewing gum (Yao et al., 2014). As discussed in section 1.4.2.5, polyols are osmotically active due to their small size and a mannitol challenge increased small bowel water content in healthy controls and patients with IBD (Marciani et al., 2010). Large doses of sorbitol can induce laxative effects in healthy individuals (Skoog et al., 2006), and patients with active IBD may intentionally or unintentionally restrict high sorbitol foods to reduce stool frequency and consistency. This may occur simply as a result of restricted fruit and vegetable intake in IBD, which is conceivable given that 24% of patients with IBD in a large cross-sectional study reported restricting fruits and vegetables in the belief that they may induce relapse (Limdi et al., 2016) and another study revealed similar proportions of patients reporting fruits and vegetables to worsen GI symptoms (23% and 26% of patients, respectively) (Prince et al., 2011). Alternatively, lower sorbitol intake in IBD may relate to a lower intake of sweetened products such as sugar-free chewing gum and low-calorie products. In active IBD particularly, a more plain and monotonous diet may be observed as a result of significant GI symptoms. However, this is speculative and investigation into dietary patterns in active IBD and inactive IBD compared to healthy controls is needed to clarify this.

There was no statistical difference in total FODMAP intakes across the groups, although the difference approached significance. The numerically lowest total FODMAP intake was in active IBD, followed by inactive IBD with FGS, inactive IBD without FGS and highest in healthy controls, although again are not statistically significantly different at this stage of recruitment. This pattern of intakes was maintained when expressed per 1000 kcal of energy intake, suggesting it was not a consequence of reduced overall food intake in active IBD. If this represents habitual intake in IBD, there could be ramifications of this. Firstly, fructans and GOS are considered
prebiotic carbohydrates (Gibson et al., 2017) and it is clear from fructan and GOS supplementation studies and low FODMAP diet studies that they have a profound impact upon the GI microbiota composition (Staudacher et al., 2017b, Staudacher et al., 2012, Bennet et al., 2017, Ramirez-Farias et al., 2009, Fernando et al., 2010, Lomax et al., 2012, Ramnani et al., 2015). In view of the numerous proposed positive GI and non-GI health implications of prebiotic consumption (Roberfroid et al., 2010), it is reasonable to suspect that chronic dietary restriction could have an adverse effect on health. Secondly, there may be implications of a lower FODMAP intake on the possible efficacy of the low FODMAP diet in IBD. If a subset of patients already have low FODMAP intakes, response to a further FODMAP reduction may be less likely. However, it is unlikely that in clinical practice a patient with low FODMAP intakes at baseline would be advised to attempt a low FODMAP diet since the expected clinical benefit is low (Whelan et al., 2018).

2.6.2 Nutrient intake

Patients with inactive IBD with and without FGS had significantly lower NSP intakes, and patients with inactive IBD with FGS had lower total fibre intakes compared to healthy controls. In addition, patients with inactive IBD with FGS had lower intakes of potassium, copper, manganese, biotin and vitamin C compared to healthy controls.

Lower fibre intake in patients with inactive IBD with FGS compared to healthy controls in this study may reflect an attempt to manage functional GI symptoms. Many of the foods that patients with IBD commonly exclude are fibre-rich, for example fruits and vegetables and legumes (Limdi et al., 2016, Vagianos et al., 2016, Cohen et al., 2013, Prince et al., 2011, Holt et al., 2017), and many patients report that dietary exclusions relate specifically to GI symptoms (Prince et al., 2011, Vagianos et al., 2016). The IBS guidelines also encompass modifying fibre intake where appropriate, to control GI symptoms (McKenzie et al., 2016). Since around a third of patients with FGS fulfilled the criteria for diarrhoea-predominant IBS, it is conceivable that reduced fibre intakes may reflect an attempt to reduce stool frequency and consistency. Lower total fibre intake in patients with inactive IBD with FGS was mirrored by lower fibre intakes per 1000 kcal of energy intake, suggesting that the differences in fibre intake were not merely the result of lower overall food intake. Total fibre intake in the healthy controls was slightly higher than the general population (PHE, 2018), and this may have increased the difference between the study groups.

A reduced fibre intake in inactive IBD could bear clinical relevance. In a longitudinal survey of patients with inactive IBD, patients with CD in the highest quintile of fibre intake were less likely to experience a CD relapse, although the same association was not observed in UC (Brotherton
et al., 2016). It is possible that patients with low fibre intakes were those with more complicated IBD and therefore more likely to relapse (irrespective of fibre intake), especially since patients with prior surgery, longer duration of disease and past hospitalisation ate less fibre. However, considering the numerous other positive health implications of fibre, adequate fibre intakes should be encouraged in patients with inactive IBD unless contraindicated (for example, in patients with fibrotic strictures).

It is interesting that fibre intakes were not significantly different in patients with active IBD compared to healthy controls. Patients are often advised to reduce fibre intake during active IBD either to reduce the risk of GI obstruction in stricturing CD or simply to reduce stool frequency and GI symptoms, although there is little evidence supporting the use of a low-fibre or low-residue diet in active IBD (Lee et al., 2014a).

Alternatively, the patients with active IBD and healthy controls in this study may indeed have similar fibre intakes. Evidence for compromised fibre intake in active IBD is lacking. A cross-sectional study utilising 7-day food diaries observed no significant difference in fibre intakes between patients with active (15 g/day) and inactive IBD (18 g/day), although there was a trend towards lower fibre intake in active IBD (Aghdassi et al., 2007). A further study revealed a lower fibre intake in patients with IBD assessed to be malnourished (Lim et al., 2014), however this likely relates to the significantly lower energy intake in the malnourished group. Lower fibre intake has been observed in patients with CD receiving immunomodulating therapy (Sousa Guerreiro et al., 2007), although again a lower total energy intake in the CD group suggests the reduced fibre intake was a consequence of reduced overall food intake. In the current study energy intakes were similar across groups, and fibre intakes remained similar even when calculated per 1000 kcal/d, suggesting that differences seen here were not merely the result of lower food intake. Limiting dietary fibre is specifically clinically indicated in stricturing CD, which constitutes only a small proportion of all patients with IBD in this study, which might explain similar fibre intakes to healthy controls. Moreover, significantly fewer patients with active IBD had CD compared to those with inactive IBD with FGS. Another consideration is that the severity of active IBD in this study was mild to moderate (Falvey et al., 2015) and dietary alterations in the active IBD group may therefore have been minimal.

Several nutrients were lower in patients with inactive IBD with FGS compared to healthy controls, which may again relate to dietary restrictions implemented to control functional GI symptoms. This is the first study to investigate nutrient intakes specifically in patients with IBD with FGS, but previous studies have established inadequate micronutrient intakes in inactive IBD compared to either the intake of healthy controls or compared to national recommendations. Inadequate micronutrients may include zinc (Filippi et al., 2006), B vitamins (Filippi et al., 2006),
calcium (Geerling et al., 2000, Vagianos et al., 2007), vitamin C (Geerling et al., 2000), vitamin D (Vidarsdottir et al., 2016, Vagianos et al., 2007). That said, the GI symptoms in this group are assumed to be functional, rather than relating to active GI inflammation. Nutrient intakes in IBS appear to be similar to, or even greater than, the general population (Bohn et al., 2013a, Williams et al., 2011), despite evidence from cross-sectional studies that patients with IBS make dietary modifications (Ligaarden et al., 2012). Interestingly, an assessment of nutrient intakes in outpatients with IBD, IBS, dyspepsia and gastro-oesophageal reflux disease revealed a lower intake of several micronutrients in the non-IBD patients compared to IBD (Gee et al., 1985). Dietary intake was measured using only a 24-hour dietary recall and it is difficult to establish whether this represented long-term dietary intake, however this may provide evidence of poorer nutritional adequacy in IBS. Otherwise, there is little evidence to date that dietary restrictions relating to GI symptoms in functional bowel disorders adversely affects nutrient intakes. It is surprising that patients with active IBD did not display inadequate intakes of micronutrients. Again, this may relate to an insufficient sample size or to the mild severity of IBD in this study, or may simply indicate that not all patients with active IBD modify their diets or are able to do so without compromising nutrient intakes.

There were minor differences in the proportion of participants meeting national dietary recommendations across the groups. A notable finding was that only 19-34% of IBD patients met calcium recommendations from dietary intake compared to 72% of healthy controls. The proportion meeting calcium requirements was particularly low in patients with active IBD, and this may relate to dairy restriction, which is implemented by as many as 65% of patients with IBD, more likely in patients with active disease and with extensive UC (Holt et al., 2017, Cohen et al., 2013, Limdi et al., 2016, Prince et al., 2011, Brasil Lopes et al., 2014). Lactose intolerance is higher in IBD than the general population (Szilagyi et al., 2016) and this may be particularly prevalent in CD due to reduced lactose digestion and resulting malabsorption. When calcium requirements were set at 700 mg/d for all participants (as opposed to 1000 mg/d in IBD and 700 mg/d in healthy controls), there was no difference across groups in the proportion meeting requirements, suggesting that the higher calcium requirement in IBD (1000 mg/d) is achieved by few patients. Calcium supplements were taken by some patients and would significantly elevate dietary intake but these were not analysed since the aim was to examine nutrient intakes from food. Nonetheless, poor intestinal calcium absorption, steroid use and greater risk of osteoporosis drive the greater calcium requirements in IBD and future analyses in larger groups of patients should address the proportion of patients achieving these intakes.

Body weight and BMI were significantly higher in patients with inactive IBD with FGS compared to healthy controls, which is somewhat unexpected considering that a lower BMI has been
observed in patients with IBD (Mijac et al., 2010, Geerling et al., 2000) and patients with IBD are regarded at risk of malnutrition (Gerasimidis et al., 2011). That said, several studies have shown similar BMI in patients with IBD and healthy controls (Filippi et al., 2006, Aghdassi et al., 2007, Vagianos et al., 2007) and one study observed that the average BMI in patients with IBD was in the overweight range ($\geq 25$ kg/m$^2$) (Vidarsdottir et al., 2016). However, several studies have demonstrated altered body composition in IBD compared to healthy controls (Bryant et al., 2013), indicating that malnutrition may not be apparent from BMI alone. The difference in BMI between patients with IBD with FGS compared to healthy controls may also be explained by a lower BMI in healthy controls resulting from the recruitment methods used to identify them.

2.6.3 Food-related quality of life

Food-related quality of life was significantly lower in active IBD and inactive IBD with FGS compared to healthy controls, while FR-QOL in inactive IBD without FGS was similar to that of healthy controls. Food-related quality of life has been shown to be lower in IBD than healthy controls and patients with asthma, but no distinction was made between active and inactive IBD (Hughes et al., 2016). This is therefore the first study to demonstrate lower FR-QOL in IBD and GI symptoms (active IBD and inactive IBD with FGS) compared to those without (inactive IBD without FGS) and compared to healthy controls. There were similar scores in patients with active IBD and inactive IBD with FGS, suggesting that the origin of GI symptoms makes little difference to FR-QOL. Although periods of active IBD will likely be short-lived, FGS can persist for months or years and the individual burden of poor FR-QOL is substantial. Furthermore, certain questions in the FR-QOL questionnaire pertain to difficulty in deriving sufficient nutrients from food and mealtime interruptions by GI symptoms, therefore low FR-QOL could imply compromised nutritional intake.

Average FR-QOL score in patients with active IBD and inactive IBD with FGS in this study were comparable to those observed in the validation cohort for the FR-QOL questionnaire (64-74 vs. 90) (Hughes et al., 2016). Previous studies indicate that patients with IBD manipulate their diet to manage the condition and such dietary restrictions impact upon social interactions involving food (Jamieson et al., 2007, Daniel, 2002). Control over food intake as a means of coping with GI symptoms is also reported by patients with IBS (Fletcher and Schneider, 2006, Jamieson et al., 2007), therefore it is unsurprising that FR-QOL scores were low in patients with IBD with GI symptoms in this study.
2.6.4 Strengths and limitations

This is the first study to assess dietary intake in patients with inactive IBD with FGS compared to patients without FGS and healthy controls and is the first study to characterise habitual FODMAP intakes in active and inactive IBD compared to healthy controls. Once the target sample size is achieved, it may make an important contribution to the current literature regarding dietary intake in IBD, which is currently limited to small studies with major methodological limitations.

A major strength of this study is the thorough prospective assessment of dietary intake using 7-day food diaries. Most of the previous assessments of dietary intake in IBD have utilised either FFQ or 3-4 day food diaries, the former being associated with recall bias and overestimation of nutrient intakes and the latter potentially failing to capture sufficient day-to-day variation in dietary intake (Barclay et al., 2008, Fallaize et al., 2014, Flood et al., 2004). One study that did use a 7-day food diary to estimate nutrient intakes did not include patients with UC (Aghdassi et al., 2007). However, although participants in the current study were encouraged to record dietary intake contemporaneously, it is entirely possible that at least some participants recorded dietary intake sometime after eating due to work or other commitments. Therefore, dietary data gathered from prospective food diaries may still be susceptible to some degree of recall bias, representing a possible limitation of the dietary assessment in this study.

Furthermore, food diaries were only completed on one occasion, therefore failing to capture fluctuations in dietary intake over time and alongside changes in IBD activity. It was therefore not the aim of this study to establish whether differences in dietary intake between groups are a possible cause or consequence of the current IBD activity. A prospective longitudinal study of dietary intake in IBD would help establish dietary patterns associated with a likelihood of IBD remission or relapse, and such a study design has been used to establish an association between fibre intake and CD relapse (Brotherton et al., 2016). Moreover, participants were urged to complete the diary in a week representative of their normal routine and were encouraged not to change their habitual diet during the study, but it is possible that the dietary intake recorded does not represent normal intake in some individuals due to conscious or unconscious change in dietary intake during dietary assessment.

Weighed food diaries would enable greater accuracy of food intake than the estimated or unweighed food diaries that were used in the current study. Unfortunately, it was not feasible to provide all participants with weighing scales for this 7-day food diary and it was also anticipated that this additional participant burden would significantly hinder compliance and diary return and impact habitual intake. A comparison of weighed and unweighed food diaries revealed similar nutrient intakes with the two methods, in contrast to an overestimation of
nutrient intakes with an FFQ (Bingham et al., 2007, Bingham et al., 1995). A major issue with unweighed food diaries is the requirement for participants to estimate portion sizes, which can lead to inaccuracies in nutrient intake data. This was addressed in this study through thorough instructions provided by an experienced dietitian, and provision of food portion size photographs and other portion size aids. Food portion size photographs have been shown to improve the accuracy of portion size estimation (Turconi et al., 2005, Nelson et al., 1996).

There are a number of methods for identifying individuals misreporting dietary intake, each with strengths and limitations (Banna et al., 2017). One of the simplest methods is to exclude participants at the extremes of nutrient intake, but this fails to individualise the assessment of misreporting and is thus likely to exclude individuals with plausible intakes while including true misreporters. An alternative method, that was used in the current study, is to calculate the ratio of reported energy intake to estimated energy requirement (EI:EER) and apply cut-offs to define plausible and implausible ratios (Huang et al., 2005). This is a simple method that is specific to weight and age (since EER estimation is based upon the Henry Oxford equation for BMR (Henry, 2005) and has been shown to agree with the doubly-labelled water energy expenditure calculation when certain cut-offs are applied (Huang et al., 2005). A major limitation of this method is that the applied PAL may be incorrect, which could under or overestimate EER and incorrectly define an individual as an under-reporter or over-reporter. In this study, the same PAL was applied to all participants as information was not available regarding occupational activity levels and physical activity levels. Therefore, the estimated proportion of under-reporters, plausible reporters and over-reporters may not be accurate for all individuals. However, the proportion of under-reporters, plausible reporters and over-reporters was not significantly different across the study groups and therefore it was not anticipated that there would be a bias associated with greater misreporting in any of the study groups and, in light of this, the analysis was not adjusted for misreporting.

A major limitation of this study is the small group sample sizes, none of which reached the target number of participants (80 per group) within the time frame for the submission of this thesis. Just over a third of enrolled participants with IBD (excluding patients with IBD with FGS who had been recruited in the RCT) returned a food diary and hence a longer time is required to recruit sufficient participants to ensure food diary numbers reach the target of 80 per group. The inadequate group sizes likely limited the ability to detect differences across the groups.

The rate of food diary return in patients with IBD (excluding patients with IBD with FGS who had been recruited in the RCT) was around 35%, compared to 67% of healthy controls. This difference in return rates probably relates to the methods of recruitment. Patients with IBD were approached by the researcher in an outpatient clinic and asked to consider participating.
in the survey. The vast majority of patients expressed a willingness to participate, in line with previous studies (Gehrmann et al., 2016, Ravikoff et al., 2012). However, a 7-day food diary is time-consuming, can be inconvenient and may be challenging for patients not familiar with recording dietary intake, and these factors probably compromised food diary return.

In contrast to the patients with IBD, healthy controls approached the researcher following study advertisement and are therefore likely to represent motivated and willing individuals, who are inherently more likely to return the diary. Furthermore, healthy controls were staff and students from a University and a hospital, and overall represented individuals with a higher educational level than patients with IBD, as evidenced by the greater proportion of healthy controls with a Masters-level degree. Although there is no evidence that a higher educational level enhances food diary completion, previous studies indicate that demographic and socioeconomic factors can predict response or non-response to surveys (Mannetje et al., 2011, Boshuizen et al., 2006). However, the predictors of patients with IBD completing and returning a food diary are unknown. Survey response rates may be improved by using monetary incentives, recorded delivery, pre-notification and shortening survey length (Edwards et al., 2009). These strategies were not possible or not relevant in the current study and whether they would improve return of a food diary, as opposed to a questionnaire survey, is unknown. However, several other strategies that were used in the current study, such as follow-up contact, unconditional incentives (the offer of a nutritional report following dietary analysis), and the use of stamped rather than franked return envelopes may have contributed to diary return (Edwards et al., 2009).

The assessment of IBD activity could be viewed as both a strength and limitation of this study. Disease activity was defined using both objective (inflammatory markers, endoscopy or imaging) and subjective (clinical disease activity indices) criteria in active IBD and inactive IBD without FGS. Some patients had only recent serum CRP concentrations available and were included in the appropriate group based upon this value. This is a potential limitation of the trial since serum CRP is not specific for GI inflammation and thus, some patients in the active group may not have had GI inflammation. Furthermore, not all patients with GI inflammation have a raised CRP, meaning that some suitable patients may have been excluded, or categorised as having inactive IBD, although the measurement of clinical disease activity indices would hopefully have avoided this.

The required cut-off for faecal calprotectin was <250 μg/g for inactive IBD, based upon a previous meta-analysis (Lin et al., 2014). A more recent meta-analysis suggests this cut-off may include participants with mild disease activity, with the optimal cut-off shown to be much lower at 50 μg/g (Mosli et al., 2015). However, this is not anticipated to significantly impact the study
results since the majority of participants were enrolled based upon a recent blood CRP, which is regularly monitored in clinical practice.

This was the first study to investigate FODMAP intakes in patients with IBD, representing a strength of this study. However, the analysis is not without limitations. Firstly, although the FODMAP database is comprehensive, it is not close to containing all foods that participants consume and FODMAP composition of speciality and ethnic foods is particularly limited. This means that some foods, which may be sources of FODMAPs, are omitted from the analysis and FODMAP intakes may therefore be underestimated. Secondly, the database is based upon the analysis of Australian foods, although some have been imported from other countries for analysis, such as plant-based milks. The FODMAP content of UK fruits, vegetables and grains may vary due to different growth environments, meaning the FODMAP analysis may not be accurate for UK diets. Additionally, and related to the prior point, some foods are available in Australia and not the UK, meaning the database is not representative of a UK diet. An additional source of error is the effect food processing techniques such as pickling and canning have on FODMAP content (Tuck et al., 2018); unfortunately, the database does not contain exhaustive processing options for individual foods. Finally, the FODMAP analysis is subject to the aforementioned limitations associated with collecting food diaries, namely under or selectively reporting dietary intake.

2.6.5 Conclusion

This study is the first to investigate intakes of all FODMAPs in IBD compared to healthy controls and nutrient intakes in inactive IBD with FGS. Using a robust dietary assessment method, differences in the intake of GOS and sorbitol were observed between patients with inactive IBD with FGS and healthy controls. Total FODMAP and fructan intakes were not significantly different across the groups, although total FODMAP intake approached statistical significance. Intakes of a number of micronutrients (e.g. potassium, copper and manganese) and fibre were significantly lower in patients with inactive IBD with FGS compared to healthy controls. These findings indicate a possible relationship between FODMAPs and FGS that requires exploration to establish whether FODMAPs directly influence GI symptoms in inactive IBD. They also suggest that patients with inactive IBD with FGS may be nutritionally vulnerable and this should be considered when implementing dietary management. Food-related quality of life was impaired in patients with IBD with GI symptoms (active IBD and inactive IBD with FGS) compared to patients without GI symptoms (inactive IBD without FGS) and healthy controls. The small sample size may have limited the power to detect differences between groups. Continued recruitment
and future analysis following attainment of the target sample size may reveal further differences in FODMAP and nutrient intakes between groups.
A double-blind, placebo-controlled, randomised, cross-over, re-challenge trial to investigate the effects of fermentable carbohydrates on functional gastrointestinal symptoms in inactive IBD
3.1 Introduction

Functional gastrointestinal symptoms (FGS) are common in patients with inactive IBD (Halpin and Ford, 2012), despite minimal evidence of intestinal inflammation. These common FGS are associated with anxiety, depression and poor health-related quality of life (Simrén et al., 2002, Berrill et al., 2013, Bryant et al., 2011, Jonefjäll et al., 2016), although the direction of this relationship has not been established. Clinicians treating patients with FGS are faced with a treatment dilemma, since escalating IBD therapy would be ineffective in this context and potentially associated with unpleasant side effects. The aetiology of FGS in IBD may differ from that of GI symptoms in IBS (without IBD) given the background of an organic inflammatory disease (Quigley, 2016). Therefore, while there is evidence that dietary fibre manipulation, antispasmodics, antidepressants and fermentable carbohydrate restriction may be effective in IBS (McKenzie et al., 2016), it is unclear whether these treatment options demonstrate equal efficacy in inactive IBD.

The case-control study presented in Chapter 2 of this thesis revealed that patients with active IBD and inactive IBD with FGS have lower intakes of GOS and polyols than healthy controls, suggesting that patients with GI symptoms may modify their diets to intentionally or unintentionally reduce high FODMAP foods. Although this case-control study does not establish a direct relationship between FODMAPs and GI symptoms in IBD, it suggests that investigation is warranted to establish the effect of FODMAPs on FGS.

Trials of dietary interventions pose unique challenges compared to pharmaceutical trials. Foods are a heterogeneous mixture of nutrients and bioactive components and multicollinearity for nutrients and food components results in difficulty in interpreting the effect of dietary factors or interventions on outcomes of interest (Freudenheim, 1999, Jacobs and Steffen, 2003). This difficulty in dietary research is relevant to trials in functional bowel disorders, and is illustrated by the case of non-coeliac gluten sensitivity (NCGS). Gluten and fructans co-exist in wheat and have both been hypothesised to influence FGS (Biesiekierski and Iven, 2015). An initial trial concluded that gluten re-challenge induced symptoms in patients with NCGS following symptom control on a gluten-free diet (Biesiekierski et al., 2011a), while a subsequent RCT demonstrated that gluten alone did not induce symptoms in self-reported NCGS on the background of a low FODMAP diet (Biesiekierski et al., 2013). Indeed, a randomised placebo-controlled crossover re-challenge trial recently established that fructans, and not gluten, were responsible for GI symptoms in patients with NCGS (Skodje et al., 2017).

An additional challenge posed by trials of dietary interventions pertains to blinding both participants and study investigators to treatment allocation. A meta-analysis of irritable bowel
syndrome (IBS) treatment trials showed a pooled placebo response rate of 37.5% (Ford and Moayyedi, 2010), emphasising the importance of blinding participants in trials of functional bowel disorders. In contrast to drug trials, in which a placebo pill that appears identical to the active treatment is relatively simple to produce, it is much more challenging to design a placebo or control diet that participants cannot identify as such (Yao et al., 2013).

Therefore, improvements in GI symptoms following dietary treatments could reflect a placebo response or a response to the restriction of another food component. While randomised, placebo-controlled trials would control for the placebo response to the low FODMAP diet, it is still possible that the diet leads to changes in food components other than fermentable carbohydrates that may influence FGS. Regarding the low FODMAP diet, it is also impossible to separate effects of different fermentable carbohydrates when implementing restriction of all FODMAPs. Restriction of FODMAPs followed by isolated re-challenge with a series of pure fermentable carbohydrates and glucose (placebo) established that FODMAPs are indeed responsible for GI symptoms in IBS (Shepherd et al., 2008). Elimination and re-challenge has also established adverse effects of food components in NCGS (Biesiekierski et al., 2013, Skodje et al., 2017), chronic constipation (Carroccio and Iacono, 2006) and asthma (Baker et al., 2000). Pure forms of the food component can be used as challenges to overcome the issues relating to the complexity of whole foods and simplifies participant blinding.

Although fructans and fructose challenges have been shown to exacerbate FGS in IBS following a low FODMAP diet (Shepherd et al., 2008), and fructan supplementation exacerbated GI symptoms in active CD on the background of a habitual diet (Benjamin et al., 2011b), the effect of blinded fermentable carbohydrate challenges following symptom control with a low FODMAP diet has not thus far been investigated in IBD. A randomised, placebo-controlled, double-blinded crossover re-challenge trial was therefore designed to establish whether fermentable carbohydrates exacerbate FGS in patients with IBD in remission.

### 3.2 Aims of the re-challenge trial

The aim of this study was to conduct a randomised, double-blinded, placebo-controlled, crossover, re-challenge trial to determine whether individual fermentable carbohydrates exacerbate FGS in patients with inactive IBD.

### 3.3 Hypotheses

Null hypothesis: In patients with inactive IBD who have reported adequate relief of FGS following a low FODMAP diet, re-challenge with individual fermentable dietary carbohydrates (fructans, GOS and sorbitol), will not exacerbate FGS compared with a placebo (glucose).
Chapter 3: Re-challenge trial

Alternative hypothesis: In patients with inactive IBD who have reported adequate relief of FGS following a low FODMAP diet, re-challenge with individual fermentable carbohydrates (fructans, GOS and sorbitol), will exacerbate FGS compared with a placebo (glucose).

3.4 Methodology

3.4.1 Trial design
This was a randomised, placebo-controlled, double-blind, crossover re-challenge trial investigating the effect of a series of individual fermentable carbohydrate challenges on FGS in patients with inactive IBD on the background of a low FODMAP diet.

3.4.2 Trial sites
Trial recruitment took place at Guy's and St Thomas’ Foundation Trust and Barts Health NHS Trust, London, UK. Participants were recruited from gastroenterology and dietetic outpatient clinics and trial visits took place in either the hospital clinic or at King’s College London (KCL), Department of Nutritional Sciences.

3.4.3 Patient selection, inclusion and exclusion criteria
Potentially eligible participants were identified by screening medical notes and clinic lists and by approaching potentially suitable patients for screening after clinic appointments.

Inclusion criteria:

- Patients aged 18 years or over;
- Diagnosed with Crohn’s disease (CD) or ulcerative colitis (UC) at least six months’ prior;
- Inactive IBD as defined by Physician Global Assessment, CRP <10 mg/L and faecal calprotectin <250 µg/g in the past four weeks. This faecal calprotectin cut-off was chosen as having been shown to perform optimally in distinguishing active from inactive IBD in terms of sensitivity and specificity in a meta-analysis (Lin et al., 2014);
- Current FGS fulfilling the Rome III criteria for IBS, functional bloating or functional diarrhoea;
- A marked improvement in FGS following the low FODMAP diet. A ‘marked response’ to the low FODMAP diet was defined as an affirmative answer to the Global Symptom Question (GSQ) “Do you currently have adequate relief of your gut symptoms?”, which patients were asked after at least two weeks on the diet.

Exclusion criteria:

- Other significant comorbidities including diabetes, cardiovascular disease and renal failure, due to the possible impact upon diet and ability to follow a dietary intervention;
• Patients with recent changes in IBD medications, currently taking steroids or antibiotics, probiotics or prebiotics in the preceding four weeks, due to the possible confounding on GI symptom responses;
• Current stricturing CD, previous proctocolectomy or current stoma, as altered physiology may alter GI symptom responses.

### 3.4.4 Interventions

Participants were allocated to a random order of three fermentable carbohydrates and one placebo challenge. The fermentable carbohydrate challenges were fructans, GOS and sorbitol (Table 3.1). Glucose was chosen as the placebo as it is efficiently absorbed in the proximal small intestine, thus does not generate a breath hydrogen rise or an increase in small intestinal water or colonic gas, and therefore should not induce GI symptoms (Major et al., 2017, Murray et al., 2014). The FODMAPs were chosen as these had not previously been investigated in FGS induction (GOS, sorbitol) or had not been investigated in FGS in IBD specifically (fructans, sorbitol). Although fructose has not been investigated as an inducer of FGS in IBD specifically, it is well established that fructose malabsorption and intolerance is present in a significant proportion of patients with functional bowel disorders (Gibson et al., 2007). Therefore, since this trial already included four carbohydrate challenges, fructose was omitted in the interest of minimising participant burden (i.e. the trial would become larger) and maximising participant retention (particularly crucial in a crossover trial).

### Table 3.1 Carbohydrate challenges and daily dose in the re-challenge trial

<table>
<thead>
<tr>
<th>Challenge</th>
<th>Dose/day</th>
<th>Composition, molecular weight</th>
<th>Product, manufacturer, country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructans</td>
<td>12 g</td>
<td>95% oligofructose, average DP 4, 666.58 g/mol</td>
<td>Orafti P95, Beneo, Germany</td>
</tr>
<tr>
<td>Alpha-GOS</td>
<td>6 g</td>
<td>Stachyose ≥55-65%, raffinose ≥14-18%, verbascose ≥16-20%; molecular weight 738.64 g/mol</td>
<td>Soybean oligosaccharide, Hunan NutraMax Inc, China</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>6 g</td>
<td>98% sorbitol, 182.17 g/mol</td>
<td>Neosorb P60W Roquette Pharma, France</td>
</tr>
<tr>
<td>Glucose (placebo)</td>
<td>12 g</td>
<td>100% dextrose monohydrate, 180.16 g/mol</td>
<td>Dextrose, Thornton and Ross, UK</td>
</tr>
</tbody>
</table>

GOS, galacto-oligosaccharides

The aim was to challenge patients with the highest possible dose of fermentable carbohydrate that could realistically be achieved in a normal UK diet and therefore demonstrate the effects of the carbohydrates when consumed within a diet (Table 3.2). The challenges were the upper end of the quantities likely to be consumed in a normal diet as participants were also following a strict low FODMAP diet during the 4-week re-challenge trial. Low FODMAP dietary advice has previously resulted in a reduction of total FODMAP intake of around 12 g/day (Staudacher et al., 2014).
Therefore, the addition of 12 or 6 g/d of fermentable carbohydrate in the form of pure challenges is likely to return the total FODMAP intake to an average daily UK intake.

Table 3.2 Estimated typical daily intakes of fermentable carbohydrates included in re-challenge trial

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Estimated intake, g/day</th>
<th>Country</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructans</td>
<td>Median 2.6 (range 1.04-4.46)</td>
<td>USA</td>
<td>(Moshfegh et al., 1999)</td>
</tr>
<tr>
<td></td>
<td>Mean 3.6 (95% CI 2.9-4.2)</td>
<td>UK (IBS)</td>
<td>(Staudacher et al., 2012)</td>
</tr>
<tr>
<td></td>
<td>Mean 4.0 (SD 1.3)</td>
<td>UK</td>
<td>(Dunn et al., 2011)</td>
</tr>
<tr>
<td></td>
<td>Median 3.9 (IQR 2.1)</td>
<td>UK</td>
<td>(Anderson et al., 2015)</td>
</tr>
<tr>
<td>GOS</td>
<td>Mean 2.0 (95% CI 1.4-2.5)</td>
<td>UK (IBS)</td>
<td>(Staudacher et al., 2012)</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>Mean 0.4 (95% CI 0.2-0.5)</td>
<td>UK (IBS)</td>
<td>(Staudacher et al., 2012)</td>
</tr>
</tbody>
</table>

The fermentable carbohydrate doses capable of inducing GI symptoms were also chosen based upon previous GI symptom provocation and supplementation studies in patients with functional bowel disorders or IBD (Table 3.3). Although the sorbitol dose for this trial is greater than the average UK intake, previous symptom provocation studies have mainly used doses of 5-10 g, and a previous study has demonstrated tolerance to a much higher dose of sorbitol, albeit in healthy volunteers (Skoog et al., 2006).
### Table 3.3 Mechanistic or prebiotic supplementation studies in functional bowel disorders or IBD using the fermentable carbohydrates included in re-challenge trial

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Outcomes assessed</th>
<th>Participants</th>
<th>Daily dose(s)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructans</td>
<td>Fructose and inulin on small intestinal water and colonic gas volume</td>
<td>Healthy volunteers</td>
<td>40</td>
<td>(Murray et al., 2014)</td>
</tr>
<tr>
<td></td>
<td>Fructose and inulin on small intestinal water and colonic gas volume</td>
<td>Healthy volunteers and IBS</td>
<td>40</td>
<td>(Major et al., 2017)</td>
</tr>
<tr>
<td></td>
<td>Fructose and fructan challenges on GI symptoms following FODMAP restriction</td>
<td>IBS</td>
<td>7, 14, 19</td>
<td>(Shepherd et al., 2008)</td>
</tr>
<tr>
<td></td>
<td>Short-chain fructo-oligosaccharides on rectal hypersensitivity</td>
<td>IBS</td>
<td>5</td>
<td>(Azpiroz et al., 2017)</td>
</tr>
<tr>
<td></td>
<td>Oligofructose on GI symptoms, stool weight, transit time and breath hydrogen</td>
<td>IBS</td>
<td>6</td>
<td>(Hunter et al., 1999)</td>
</tr>
<tr>
<td></td>
<td>Fructo-oligosaccharides on GI symptoms</td>
<td>IBS</td>
<td>20</td>
<td>(Olesen and Gudmand-Hoyer, 2000)</td>
</tr>
<tr>
<td></td>
<td>Short-chain fructo-oligosaccharides on GI symptoms and quality of life</td>
<td>Functional bowel disorders</td>
<td>5</td>
<td>(Painau et al., 2008)</td>
</tr>
<tr>
<td></td>
<td>Oligofructose-enriched inulin on disease activity, FCP and GI symptoms</td>
<td>Active UC</td>
<td>12</td>
<td>(Casellas et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>Fructo-oligosaccharides on clinical disease activity, GI microbiota and immunology</td>
<td>Active CD</td>
<td>15</td>
<td>(Lindsay et al., 2006)</td>
</tr>
<tr>
<td></td>
<td>Fructo-oligosaccharides on clinical disease activity, GI microbiota and immunology</td>
<td>Active CD</td>
<td>15</td>
<td>(Benjamin et al., 2011b)</td>
</tr>
<tr>
<td></td>
<td>Oligofructose-enriched inulin on disease activity and faecal metabolite profile</td>
<td>Active CD</td>
<td>20</td>
<td>(De Preter et al., 2013)</td>
</tr>
<tr>
<td>GOS</td>
<td>Effect of trans-GOS on GI symptoms, stool output and faecal microbiota</td>
<td>IBS</td>
<td>3.5 or 7</td>
<td>(Silk et al., 2009)</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>Sorbitol and mannitol on GI symptoms and breath hydrogen</td>
<td>Healthy volunteers and IBS</td>
<td>10</td>
<td>(Yao et al., 2014)</td>
</tr>
<tr>
<td></td>
<td>Fructose, sorbitol and fructose-sorbitol mixture on GI symptoms</td>
<td>IBS, functional diarrhoea or excessive flatulence/bloating</td>
<td>5</td>
<td>(Rumessen and Gudmand-Hoyer, 1988)</td>
</tr>
<tr>
<td></td>
<td>Lactose, fructose and sorbitol on GI symptoms</td>
<td>Functional bowel disorders</td>
<td>5</td>
<td>(Fernández-Bañares et al., 1993)</td>
</tr>
<tr>
<td></td>
<td>Presence of sugar malabsorption and dietary restriction of malabsorbed sugar</td>
<td>Functional bloating</td>
<td>3.5</td>
<td>(Fernandez-Banares et al., 2006)</td>
</tr>
</tbody>
</table>

GOS, galacto-oligosaccharides; GI, gastrointestinal; FCP, faecal calprotectin; IBS, irritable bowel syndrome; UC, ulcerative colitis; CD, Crohn’s disease
Chapter 3: Re-challenge trial

At the baseline visit (visit 1; prior to challenge phase), participants were given three containers for each of the three days of the challenges, each of which contained the relevant carbohydrate in powder form (i.e. 12 containers in total) (Figure 3.1). Each container was labelled with the relevant randomisation ID and the week in which it was to be consumed (for example: ‘FM01, week 1’). Participants were instructed to reconstitute the powder with 200 ml cold water and consume it with or without food before 11:00 on days 1-3 of each of the four challenge weeks (each week included the 3-day challenge and 4-day washout).

Figure 3.1 The containers containing the carbohydrate challenge powders given to participants. Each was labelled with the week of the trial in which it was to be consumed (week 1-4) and the participant ID. Since there was 3 challenge days for each carbohydrate, there were three containers per week.

3.4.5 Randomisation

Crossover trials carry the advantage that each participant receives all treatments or challenges, which mitigates inter-individual variability, thus increasing the statistical power of the study and allowing smaller sample sizes (Louis et al., 1984). However, crossover trials are susceptible to order and carryover effects which can render the results difficult to interpret. This design is suited to conditions that are relatively stable over time (van Zanten et al., 1999). Although functional bowel disorders fluctuate over time, a crossover design was most appropriate in this trial given the expected inter-individual variability in symptom responses and the infeasibly large sample size that would be required for a four-arm parallel trial. Patients were allocated to a random order of challenges to neutralise possible carryover effects. There was the potential for
a greater placebo (or 'nocebo') response during the first challenge, which could ultimately inflate (if a FODMAP is the first challenge) or suppress (if the placebo is the first challenge) the effect of the FODMAPs compared to the placebo challenge. Additionally, order effects may be large in crossover trials with shorter treatment periods (Spiller, 1999), such as in this trial.

The random order in which each participant was to consume the four challenges (fructans, GOS, sorbitol and glucose placebo) was generated using an online random number generator (http://www.randomization.com) by a member of the research team not involved in trial recruitment. Since there are only twenty-four possible permutations of the four challenges, an additional block of eight random orders was generated to maintain balance in the allocations.

3.4.6 Blinding
Maximum possible blinding of participants and researchers to treatment allocation is recommended in IBS and IBD trials to mitigate the substantial expectancy effects (placebo and nocebo) characteristic of these disorders, and to minimise conscious and unconscious bias introduced by researchers (Irvine et al., 2016, Elsenbruch and Enck, 2015). Open challenges are problematic in establishing adverse reactions due to potentially significant 'nocebo' responses (Niec et al., 1998), defined as the adverse effects experienced after an inert treatment due to negative expectations and negative experiences of previous treatments (Elsenbruch and Enck, 2015). For example, adverse reactions to glucose placebo challenges have been noted in 30-63% of patients in the context of food allergy and intolerance (Pastorello et al., 1989, Farah et al., 1985).

Although participants were aware that the purpose of this trial was to investigate the effects of fermentable carbohydrate challenges on FGS, the specific types of carbohydrates included in the challenges was not disclosed until all data had been collected and the trial had concluded. Blinding of participants was further facilitated by using opaque sealed containers for the carbohydrate challenges, thus obscuring the view of the colour or quantity of the carbohydrate powder.

Fermentable carbohydrates were weighed into the opaque containers, labelled and arranged into opaque bags for each participant by a researcher not involved in recruitment. This allowed the recruiting researchers to collect the appropriate bag upon enrolment of a new participant, thus remaining blinded to the order of challenges for each participant.

3.4.7 Compliance
Adherence to a dietary intervention clearly has a major influence over its perceived effectiveness. In this trial, participants were instructed to follow a strict low FODMAP diet but
were not provided with low FODMAP foods or meals. While controlled feeding studies (in which participants are provided with all foods to be consumed) maximise participant adherence, this design does not reflect clinical practice and is often infeasible. Additionally, the provided study diet is likely to differ substantially from the participants’ usual diet, which may heighten anxiety and stress related to perceived symptom triggers that the experimental diet may contain, potentially impacting upon symptom responses (Colagiuri, 2010).

In this study, detailed and tailored low FODMAP diet education was provided by the research dietitians. In addition, participants were contacted once weekly by the research dietitians to address any questions or concerns and to encourage compliance to both the low FODMAP diet and to the FODMAP challenges.

In the absence of biomarkers to measure adherence to the low FODMAP diet, non-validated patient-reported compliance has mainly been assessed in trials investigating this diet (Halmos et al., 2014b, Staudacher et al., 2012). In this trial, adherence to the low FODMAP diet was assessed on days 1-3 of each challenge week (i.e. on the days that challenge drinks were consumed) with the question “How much of the time today did you follow the low FODMAP diet?” with five possible responses: “never”, “a quarter of the time”, “half of the time”, “three quarters of the time”, or “always”. Participants also recorded intake of challenge drinks on days 1-3 of each challenge period by answering the question “How much of the test drink did you consume?” with five possible responses: “none”, “less than half”, “half of it”, “more than half but not all” or “all”. The above written compliance questions were recorded in a paper diary provided to participants at visit 1 (prior to challenges).

3.4.8 Adverse events and withdrawals
Researchers enquired about adverse events during weekly telephone contacts conducted during the trial. The nature, duration, severity and relation of any adverse events to the challenge drinks were recorded consistently on a pre-designed form.

3.4.9 Ethics approval
All participants provided informed consent prior to providing stool samples or before any trial procedures. The study was approved by London - Camberwell St Giles Research Ethics Committee (reference number 15/LO/1878). The study was approved by the respective research and development offices prior to patient recruitment.
3.4.10 Trial protocol and procedures

The trial protocol and procedures are illustrated in Figure 3.2.

3.4.10.1 Screening

There were two potential streams of participants:

a) Retrospective participants: patients who had previously received low FODMAP dietary advice as part of routine clinical care, with a resulting GI symptom improvement

b) Prospective participants: patients referred to the dietetic department or directly to the research dietitians for dietary advice for FGS were given low FODMAP advice by the researchers and monitored for a response

All researchers actively recruiting patients (SC, thesis author; Alexis Prince, MRes Student; Clio Myers, MRes Student) were specialised and experienced in delivering low FODMAP dietary advice in clinical practice. Prospective participants met with the researchers at either the hospital clinic or King’s College London for a dietetic consultation lasting 45-60 minutes, during which the low FODMAP diet was discussed in detail and appropriate written resources were provided. Patients were asked to restrict all fermentable carbohydrates, including fructans, GOS, sorbitol, mannitol, lactose and excess fructose. Patients were contacted once a week to assess response to the diet. Patients achieving adequate relief of GI symptoms within four weeks on the low FODMAP diet, and continuing to meet all other inclusion criteria, underwent further screening for eligibility in the study by providing a blood and stool sample for CRP and faecal calprotectin (section 3.4.3), respectively, prior to enrolment in the re-challenge trial.

Retrospective participants were identified through retrospective screening of gastroenterology and dietetic outpatient clinic lists at the hospital sites. Patients with IBD who had received low FODMAP dietary advice for FGS and reportedly experienced a marked improvement in GI symptoms were approached via telephone to discuss the re-challenge trial. Patients meeting all inclusion criteria and none of the exclusion criteria and expressing an interest in participating attended a screening visit with the researchers. During this visit, they were instructed to recommence a strict low FODMAP diet until adequate relief of FGS was achieved. Patients then provided a blood sample for CRP and a stool sample for faecal calprotectin screening. Figure 3.2 depicts the trial procedures and visits.

3.4.10.2 Visit 1

Patients with acceptable concentrations of CRP and faecal calprotectin (section 3.4.3) and reporting adequate relief of GI symptoms following a low FODMAP diet returned to the hospital clinic or King’s College London for a trial baseline visit, during which they were consecutively
assigned a unique randomisation code from FM01-FM32 corresponding to a random order of a series of fermentable carbohydrate challenges (section 3.4.6 for randomisation; section 3.4.4 for interventions). Participants completed the GSQ, Gastrointestinal Symptom Rating Scale (GSRS) and the Bristol Stool Form Scale (BSFS), with reference to the preceding 7 days (section 3.4.12). Participants were provided with an opaque bag containing the challenge carbohydrates (section 3.4.7), and a stool, symptom and compliance diary for the 4-week re-challenge trial, along with detailed instructions regarding when and how to complete the challenges. Participants were instructed to continue a strict low FODMAP diet throughout the re-challenge trial.

3.4.10.3 Weekly monitoring

Participants were contacted once per week during the challenge phase the day before commencing each challenge (day 0) to ensure they had adequate relief, to address any questions or concerns and to monitor adverse events (section 3.4.9).
Figure 3.2 Flow diagram showing the re-challenge trial procedure. FCP, faecal calprotectin
3.4.10.4 Visit 2

Following completion of all four challenges and the final washout period, participants returned to the hospital clinic or King’s College London for an end of trial visit with the researchers. Symptom, stool and adherence diaries were checked for completeness and participants repeated the GSQ, GSRS and BSFS. Participants provided a stool and blood sample for faecal calprotectin and CRP determination. Prospective participants were provided with FODMAP reintroduction advice as part of routine clinical care.

3.4.11 Outcome measures and rationale

3.4.11.1 Gastrointestinal symptoms

The primary outcome was adequate relief of FGS on the final day of the 3-day challenges measured using the GSQ, “Do you currently have adequate relief of your gut symptoms?”

The concept of ‘adequate relief’ was proposed as an outcome in IBS drug trials to overcome the difficulties posed by the heterogeneity of IBS symptoms. This measure was believed to be clinically relevant and to represent the overall experience of GI symptoms and well-being (Mangel et al., 1998). At the time of planning the current trial, the Rome III guidelines recommended binary end-points as outcome measures in FGID trials (Irvine et al., 2006) and therefore the GSQ was chosen as the primary outcome.

Functional GI symptoms are extremely heterogeneous between patients and therefore it is advised that in addition to a subjective global assessment of symptoms (e.g. GSQ), a scale which measures individual symptom incidence and severity should also be administered (Irvine et al., 2006). Individual symptom incidence and severity was assessed in this study using the GSRS, composed of 12 symptoms including abdominal pain, bloating, and flatulence. This is a reproducible four-point Likert scale (0 none/absent; 1 mild, 2 moderate, 3 severe) that has been validated for use in IBS (Svedlund et al., 1988).

3.4.11.2 Stool output

The BSFS was completed prospectively on the day prior to each challenge (day 0) and on each of the three challenge days (days 1-3) of each of the four challenge periods. This simple measure rates stool form on a scale from type 1 (hardest stool form) to 7 (softest stool form) and is widely used in research and clinical practice. The Rome Foundation recommends the use of the BSFS in the diagnosis of functional bowel disorders and IBS subtype classification (Mearin et al., 2016). Changes in stool form as measured by the BSFS correlate well with changes in whole-gut transit time (Lewis and Heaton, 1997b) and with stool water content (Blake et al., 2016). The scale
demonstrates substantial validity and reliability and is able to detect differences in stool form between healthy individuals and patients with IBS-D (Blake et al., 2016).

**3.4.11.3 Biomarkers**

The aim of this trial was to investigate the effect of fermentable carbohydrate challenges on FGS in patients with inactive IBD, and therefore it was essential to establish that GI symptoms were not related to active GI inflammation. Although endoscopy and/or imaging are preferable for establishing IBD activity, these procedures are invasive and costly. Serum and stool biomarkers provide non-invasive, yet objective, measures of GI inflammation and can therefore facilitate assessment of patients for clinical trial recruitment where endoscopy and imaging may not be feasible or appropriate (Peyrin-Biroulet et al., 2015).

Faecal calprotectin correlates with endoscopic disease activity in both CD and UC, suggesting it to be a surrogate marker of GI inflammation (Arai et al., 2017, Schoepfer et al., 2013). It is therefore increasingly used to monitor disease activity in established IBD (Lehmann et al., 2015). Although faecal calprotectin thresholds for defining IBD activity are unclear, a ≤250 µg/g cut-off provided optimal sensitivity (80%) and specificity (82%) for detecting endoscopically inactive IBD in a meta-analysis of diagnostic accuracy studies (Lin et al., 2014). Lower cut-offs (50 µg/g and 100 µg/g) compromised specificity (60% and 66%, respectively), which in practice would increase the risk of obtaining false positives (i.e. inactive IBD incorrectly classified as active IBD and therefore unnecessarily excluded from the trial). A faecal calprotectin of <250 µg/g was therefore required for inclusion in this trial.

Patients provided stool samples prior to randomisation (at screening, to determine eligibility for inclusion) and at end of trial (visit 2). For convenience, patients were permitted to collect stool samples and store them in a refrigerator for up to 6 days in advance of providing them to researcher, as per the manufacturers’ instructions. Faecal calprotectin concentration was quantified by trained researchers using the Bühlmann Quantum Blue® lower range quantitative immuno-chromatographic assay.

This test is a lateral flow immunoassay followed by calprotectin quantitation by a reader designed to analyse colorimetric tests, with an upper limit of 300 µg/g. Lateral flow assays have numerous diagnostic uses within healthcare, particularly where a rapid and simple test is required (Koczula and Gallotta, 2016).
This technique permitted calprotectin quantification by the researchers shortly after the screening visit, thus enabling efficient recruitment. In contrast, calprotectin ELISA requires at least 3 hours to complete, is more technically demanding, and the microplate layout and cost makes it suited to analysing samples in batch (e.g. upon completion of a trial) rather than on an individual basis (Labære et al., 2014). Although the rapid technique does not replace ELISA for quantification of calprotectin, it reduces processing time significantly and performs comparably in the investigation of suspected or known IBD (Labære et al., 2014), particularly at lower faecal calprotectin concentrations (Kolho et al., 2012).

The lateral flow immunoassay is based upon the movement of a liquid sample (containing the analyte of interest; in this case, calprotectin) via capillary force along a membrane, containing various regions to which molecules able to interact with the analyte are attached (Posthuma-Trumpie et al., 2009, Koczula and Gallotta, 2016) (Figure 3.3). The diluted faecal sample is loaded onto the sample pad (depicted by ‘S’ in Figure 3.4B) and migrates to the conjugate release pad which is coated with monoclonal anti-calprotectin antibodies conjugated to a coloured/fluorescent label. These antibodies bind to calprotectin in the sample and the new calprotectin-antibody conjugates migrate towards the test line. This porous membrane contains monoclonal antibodies to the calprotectin-antibody conjugates, and colour is generated upon binding. The signal intensity of the test line is measured by the POC reader to determine calprotectin concentration in the sample.
The calprotectin extraction and assay were carried out as per the manufacturers’ instructions (Alpha Laboratories):

1. The dosing tip of the extraction device was removed (figure 3.4A) and inserted into the stool sample. This was repeated 3-5 times until all grooves were filled with stool. The dosing tip was inserted back into tube containing buffer.
2. The sample was homogenised by vortexing the extraction device for 30 seconds. The sample was then incubated upright at room temperature for 10 minutes. This was repeated as necessary to remove all stool from dosing tip.
3. 300 µl buffer was transferred into a test tube, along with 20 µl diluted stool from extraction device. This mixture was vortexed briefly.
4. The sample was incubated upright at room temperature for 10 minutes.
5. 60 µl diluted faecal extract was transferred onto the sample loading port of the test cassette (figure 3.4B)
6. The test cassette was incubated for 12 minutes at room temperature.
7. The test cassette was placed into the test cassette drawer of the Quantum Blue® reader and the sample was read as per the manufacturer’s instructions.

Blood samples were also collected by nurses or the lead researcher at baseline and end of trial for CRP quantification. As patients were identified from gastroenterology clinics and required this test as part of routine clinical care, this was carried out in the respective hospital pathology laboratories using a standard assay.
Patients were required to have a CRP <10 mg/L for inclusion in the study (section 3.4.3). Although 5 mg/L is more commonly used as a cut-off for defining IBD activity (Walsh et al., 2016), a mildly raised CRP is not specific to GI inflammation, and 0-10 mg/L is the normal reference range for CRP in the local hospital laboratories. The slightly higher threshold was used to avoid unnecessary patient exclusions resulting from a mildly raised CRP (5-10 mg/L), which could be unrelated to GI inflammation.

3.4.12 Sample size calculation
The sample size calculation was based on the primary outcome, which was the proportion of patients reporting adequate relief of GI symptoms on the final day of each challenge. The estimation of the effect size of the challenges was based on the only trial of this nature, in which patients with IBS consumed a series of pure fermentable carbohydrate challenges on the background of a strict low FODMAP diet (Shepherd et al., 2008). During the 19 g/d fructan challenge, 77% of participants reported that their symptoms were not adequately controlled, compared to 14% during the glucose (placebo) challenge. Since the doses of fructans chosen for the current study (12 g/d) were lower than those in the IBS re-challenge trial (19 g/d), it was anticipated that the effect size would be lower. Therefore, it was estimated that in the current study, 50% of participants would not have adequate relief of GI symptoms during the fructan challenge, compared to 14% during the placebo challenge.

Assuming a power of 80% and a 2-sided significance of 5%, an estimated 26 participants would be required to detect a difference in the primary outcome. The sample size calculation can be seen below, calculated by hand using a formula for a primary comparison of proportions:

Calculating the standardised difference $\Delta = \frac{P_1 - P_2}{\sqrt{\bar{p} \times (1-\bar{p})}}$ where $\bar{p} = \frac{P_1 + P_2}{2}$

$$\Delta = \frac{0.50 - 0.14}{\sqrt{0.32 \times (1 - 0.32)}}$$

$$\Delta = 0.7717436331$$

Using values for a significance level of 5%, $z \left(1 - \frac{\alpha}{2}\right) = 1.96$, and a power of 80%, $z(1 - \beta) = 0.8416$ then:

$$m = \frac{2 \times [1.96 + 0.8416]^2}{\Delta^2}$$

$$m = \frac{15.6979}{0.7717436331 \times 0.7717436331}$$

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\[ m = 26.365696791 \]

= 26 participants per treatment group

Based on the previous study of this nature (Shepherd et al., 2008), an attrition rate of 15% was assumed and an additional five participants would be required to ensure that the study was adequately powered at 80%. Therefore, the aim was to recruit 32 participants in total.

3.4.13 Statistical analysis

Following data collection for all 32 participants, data was entered into an excel spreadsheet by the author of this thesis (SC). All statistical analysis was performed using IBM SPSS Statistics for Windows Version 22.0. Un-blinding of the challenge order allocation was performed after analysis of the primary endpoint.

All continuous data was examined for normality prior to choosing statistical tests. Any non-normally distributed data were analysed using non-parametric tests. For demographic data, continuous variables were compared between participants with CD and UC using independent t-tests or a Mann-Whitney U test, while categorical variables were compared using a Chi-squared test.

When comparing continuous variables across the carbohydrate challenges, a one-way repeated-measures analysis of variance (ANOVA) was used, or a Friedman’s test for non-parametric data. Following a significant difference across groups, a paired t-test or Wilcoxon Signed-Rank test was used to compare between two challenges. A chi-squared test or Friedman’s test was used to compare categorical variables across the challenges. A paired t-test or Wilcoxon Signed-Rank test was used to compare continuous variables between the start and end of the trial (CRP and faecal calprotectin concentrations). A Chi-squared test was used to compare categorical variables between two challenges. A Bonferroni correction was applied where multiple challenges were compared.

3.4.14 Roles and responsibilities

Patients were recruited by AP (n=10), CM (n=14) and SC (n=8). Each researcher organised CRP and faecal calprotectin quantification and conducted all trial visits for each patient screened/recruited. All researchers promoted the trial through increasing awareness among gastroenterologists and dietitians and encouraging referrals to the trial. This was achieved through attendance and oral communication at gastroenterology research meetings and weekly
outpatient clinics and liaising regularly with gastroenterology dietitians. Data entry, analysis and interpretation, writing of the paper (Cox et al., 2017) and thesis chapter was undertaken by SC.

3.5 Results: the effect of fermentable carbohydrate challenges on gastrointestinal symptoms and inflammatory markers in inflammatory bowel disease

The following section describes the results of a randomised, placebo-controlled, double-blind, crossover re-challenge trial, designed to investigate whether individual fermentable carbohydrates exacerbate FGS in patients with inactive IBD.

3.5.1 Patient recruitment, characteristics, compliance and adverse events

3.5.1.1 Patient recruitment and progress

The randomised controlled trial opened for recruitment in March 2014 and was completed in September 2015, recruiting thirty-two participants in 18 months.

Between March 2014 and October 2015, 293 patients were screened for eligibility. Thirty-two patients were recruited (CD, n=14; UC, n=18). The main reasons for screening failures were evidence of active IBD, a current stricture in patients with CD and significant comorbidities, including diabetes and cardiovascular disease. Trial data in accordance with CONSORT requirements are shown in Figure 3.5. Twenty-six participants were recruited from Guy’s and St Thomas' NHS Foundation Trust and six from Barts Health NHS Trust.
Figure 3.5 CONSORT diagram for the re-challenge trial. FCP, faecal calprotectin; FGS, functional gastrointestinal symptoms; CD, Crohn’s disease; UC, ulcerative colitis.
Three participants exited the trial prematurely. Two were withdrawn after randomisation but prior to consuming the first challenge; one due to personal commitments, and one had a second blood test revealing a CRP exceeding 10 mg/L. One participant was withdrawn prior to commencing the final challenge due to developing pneumonia and commencing a course of antibiotics. Trial data were not available for these participants. Trial data for all challenge arms were available for all remaining 29 participants (CD, n=12; UC, n=17). Baseline demographic characteristics and disease phenotype for these participants are provided in Table 3.4.
Table 3.4 Baseline demographic characteristics of the participants

<table>
<thead>
<tr>
<th></th>
<th>Total IBD (n=29)</th>
<th>Crohn’s disease (n=12)</th>
<th>Ulcerative colitis (n=17)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, n (%)</td>
<td>11 (34.4)</td>
<td>4 (33.3)</td>
<td>7 (41.2)</td>
<td>0.668^c</td>
</tr>
<tr>
<td>Age (y), median (IQR)</td>
<td>39.0 (19.5)</td>
<td>40.5 (25.0)</td>
<td>32.0 (19.5)</td>
<td>0.661^b</td>
</tr>
<tr>
<td>Disease duration (y),</td>
<td>12.8 (13.0)</td>
<td>15.6 (15.8)</td>
<td>10.9 (10.6)</td>
<td>0.346^b</td>
</tr>
<tr>
<td>mean (SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time since last flare,</td>
<td></td>
<td></td>
<td></td>
<td>0.060^a</td>
</tr>
<tr>
<td>n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤1 year</td>
<td>15 (46.9)</td>
<td>3 (25.0)</td>
<td>12 (70.6)</td>
<td></td>
</tr>
<tr>
<td>1-5 years</td>
<td>8 (25.0)</td>
<td>6 (50.0)</td>
<td>2 (11.8)</td>
<td></td>
</tr>
<tr>
<td>6-10 years</td>
<td>3 (9.4)</td>
<td>1 (8.3)</td>
<td>2 (11.8)</td>
<td></td>
</tr>
<tr>
<td>&gt;10 years</td>
<td>3 (9.4)</td>
<td>2 (16.7)</td>
<td>1 (5.9)</td>
<td></td>
</tr>
<tr>
<td>Montreal classification</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Location</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L1, ileal: 3 (25)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L2, colonic: 5 (42)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L3, ileocolonic: 4 (33)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Behaviour</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1, nonstricturing,</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nonpenetrating: 10 (83)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B2, stricturing: 0 (0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B3, penetrating: 2 (17)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P, perianal disease:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 (17)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP (mg/L), mean (SD)</td>
<td>2.4 (1.7)</td>
<td>2.3 (1.3)</td>
<td>2.5 (2.0)</td>
<td>0.741^b</td>
</tr>
<tr>
<td>FCP (µg/g), mean (SD)</td>
<td>30.2 (29.9)</td>
<td>19.8 (12.2)</td>
<td>37.6 (36.3)</td>
<td>0.116^b</td>
</tr>
<tr>
<td>Disease activity score,</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBI score 2.3 (3.3)</td>
<td></td>
<td></td>
<td>SCCAI score 1.8 (1.9)</td>
<td>-</td>
</tr>
<tr>
<td>FBD category, n (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.596^a</td>
</tr>
<tr>
<td>IBS</td>
<td>12</td>
<td>4</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Functional bloating</td>
<td>12</td>
<td>5</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Functional diarrhoea</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Previous surgery, n (%)</td>
<td>2 (6.3)</td>
<td>2 (16.7)</td>
<td>0 (0)</td>
<td>-</td>
</tr>
<tr>
<td>Medications, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-ASA</td>
<td>18 (56.3)</td>
<td>3 (25.0)</td>
<td>15 (88.2)</td>
<td>0.002^a</td>
</tr>
<tr>
<td>Thiopurines</td>
<td>10 (31.3)</td>
<td>6 (50.0)</td>
<td>4 (23.5)</td>
<td>0.280</td>
</tr>
<tr>
<td>Biologics</td>
<td>3 (9.4)</td>
<td>3 (25.0)</td>
<td>0 (0)</td>
<td>0.119</td>
</tr>
</tbody>
</table>

^a Chi-squared test; ^b Unpaired t-test; ^c CD vs. UC

FCP, faecal calprotectin; FBD, functional bowel disorder; IBS, irritable bowel syndrome; 5-ASA, 5-aminosalicylates

3.5.1.2 Baseline patient demographic and clinical characteristics

Participants were aged 22-69 years (median 39 years, IQR 19.5), and 11 (34.4%) were male. All participants met Rome III criteria for either IBS (12/29, 41.4%), functional bloating (12/29, 41.4%) or functional diarrhoea (5/29, 17.2%). Mean CRP was 2.4 (1.7) mg/L and faecal calprotectin was 30.2 (29.9) µg/g at baseline. Except for a significantly greater proportion of patients with UC taking 5-ASA (15/17, 88.2% vs. 3/12, 25.0%, p=0.002), patients with CD and UC
were similar in baseline characteristics (Table 3.4). There were no significant differences in symptom incidence and severity at baseline (measured at visit 1) between CD and UC (Table 3.5). The most commonly experienced symptoms prior to randomisation were abdominal bloating (13/29, 45%), flatulence (16/29, 55%), incomplete evacuation (13/29, 45%) and tiredness (24/29, 83%).

Table 3.5 Gastrointestinal Symptom Rating Scale scores at randomisation

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Total IBD (n=29)</th>
<th>Crohn’s disease (n=12)</th>
<th>Ulcerative colitis (n=17)</th>
<th>P-valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain</td>
<td>0.38 (0.50)</td>
<td>0.42 (0.52)</td>
<td>0.35 (0.50)</td>
<td>0.739</td>
</tr>
<tr>
<td>Bloating</td>
<td>0.45 (0.51)</td>
<td>0.42 (0.51)</td>
<td>0.47 (0.51)</td>
<td>0.783</td>
</tr>
<tr>
<td>Flatulence</td>
<td>0.59 (0.57)</td>
<td>0.67 (0.65)</td>
<td>0.53 (0.51)</td>
<td>0.531</td>
</tr>
<tr>
<td>Belching</td>
<td>0.24 (0.58)</td>
<td>0.42 (0.79)</td>
<td>0.12 (0.33)</td>
<td>0.239</td>
</tr>
<tr>
<td>Gurgling</td>
<td>0.41 (0.50)</td>
<td>0.33 (0.49)</td>
<td>0.47 (0.51)</td>
<td>0.478</td>
</tr>
<tr>
<td>Urgency</td>
<td>0.52 (0.69)</td>
<td>0.42 (0.67)</td>
<td>0.59 (0.71)</td>
<td>0.518</td>
</tr>
<tr>
<td>Incomplete evacuation</td>
<td>0.52 (0.63)</td>
<td>0.58 (0.52)</td>
<td>0.47 (0.72)</td>
<td>0.645</td>
</tr>
<tr>
<td>Nausea</td>
<td>0.24 (0.51)</td>
<td>0.25 (0.62)</td>
<td>0.24 (0.44)</td>
<td>0.941</td>
</tr>
<tr>
<td>Heartburn</td>
<td>0.07 (0.37)</td>
<td>0.17 (0.58)</td>
<td>0.00 (0.00)</td>
<td>0.339</td>
</tr>
<tr>
<td>Acid regurgitation</td>
<td>0.10 (0.41)</td>
<td>0.17 (0.58)</td>
<td>0.06 (0.24)</td>
<td>0.495</td>
</tr>
<tr>
<td>Lethargy</td>
<td>1.21 (0.82)</td>
<td>1.33 (0.65)</td>
<td>1.12 (0.93)</td>
<td>0.495</td>
</tr>
<tr>
<td>Overall</td>
<td>0.55 (0.51)</td>
<td>0.67 (0.49)</td>
<td>0.47 (0.51)</td>
<td>0.313</td>
</tr>
</tbody>
</table>

a 0=absent, 1=mild, 2=moderate, 3=severe; b CD vs UC; unpaired t-test

3.5.1.3 Compliance

Data on adherence to the low FODMAP diet and the challenge drinks were available for all 29 participants throughout all challenges and data were included in the analysis for all patients completing the trial irrespective of compliance to the low FODMAP diet and the challenge drinks.

One participant reported consuming less than half of the drink on the first two days of the GOS challenge, and half of the drink on the remaining day. One participant did not consume any of the drink on day 3 of the glucose challenge due to experiencing “unbearable symptoms”. The numbers of participants consuming all of the carbohydrate drink on all three days of the challenge was high during the fructan (97%), GOS (93%), sorbitol (100%), and glucose (97%) challenges (P=0.392).

All participants reported good adherence to the low FODMAP diet during all of the challenge periods (defined as ≥75% adherence on all three challenge days). There was no significant
difference in the proportion of participants reporting full adherence to the low FODMAP diet across the challenges, assessed using a Friedman’s test ($P=0.065$).

### 3.5.1.4 Adverse events

Two adverse events were reported during the trial. One participant reported dizziness and severe faecal incontinence during the fructan challenge, which resolved on the same day. This was not thought to require further investigation and the participant chose to continue the trial. One patient developed pneumonia following the GOS challenge, not thought relate to the challenge drinks, and was withdrawn due to having a course of antibiotics.

### 3.5.2 Gastrointestinal symptoms

Across the challenges, there was a significant difference in the proportion of participants reporting adequate relief of GI symptoms on the final day (‘day 3’) of the challenges ($P=0.033$) (Table 3.6). Post hoc correction comparing differences between each FODMAP challenge and glucose (placebo) revealed significantly fewer participants reported adequate relief on the final day of the fructan challenge (18/29, 62.1%) than the glucose challenge (26/29, 89.7%) ($P=0.033$) but no significant differences were found between GOS (22/29, 75.9%, $P=0.306$) or sorbitol (23/29, 79.3%, $P=0.771$) compared to glucose. The difference in the incidence (number of days) of adequate relief across the challenges approached significance ($P=0.057$), with the lowest incidence being for fructans (Table 3.6).
Table 3.6 Adequate relief during and on the final day of the challenges

<table>
<thead>
<tr>
<th></th>
<th>Fructan</th>
<th>GOS</th>
<th>Sorbitol</th>
<th>Glucose</th>
<th>P value</th>
<th>P value for challenge vs glucose&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fructan</td>
</tr>
<tr>
<td>Participants with</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.033&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>adequate relief, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>On final day of</td>
<td>18 (62.1)</td>
<td>22 (75.9)</td>
<td>23 (79.3)</td>
<td>26 (89.7)</td>
<td>0.033&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>challenge</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.033</td>
</tr>
<tr>
<td>On all three days of</td>
<td>15 (51.7)</td>
<td>20 (69.0)</td>
<td>21 (72.4)</td>
<td>23 (79.3)</td>
<td>0.070&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>challenge</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of days of</td>
<td>2.1 (1.2)</td>
<td>2.4 (1.0)</td>
<td>2.5 (1.0)</td>
<td>2.7 (0.8)</td>
<td>0.057&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>adequate relief</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>during challenge, mean (SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Friedman’s test across the challenges; <sup>b</sup> Repeated-measures ANOVA across the challenges; <sup>c</sup> Each challenge compared with placebo (glucose) using Wilcoxon Signed Rank test with Bonferroni correction for multiple comparisons

GOS, galacto-oligosaccharides; SORB, sorbitol

There were significant differences in the incidence (number of days) of moderate or severe pain (P=0.006), bloating (P<0.001), flatulence (P=0.001) and overall symptoms (P=0.021) across the challenges (Table 3.7). Following post hoc correction, there was a significantly greater incidence of moderate or severe pain (P=0.014), bloating (P=0.017) and flatulence (P=0.034) during the fructan challenge compared to the glucose, but not for any of the other challenges (Table 3.7).

There were significant differences in the average (3-day) severity of pain (P=0.004), bloating (P<0.001), flatulence (P<0.001), incomplete evacuation (P=0.013) and lethargy (P=0.012) across the challenges. Following post hoc correction, there was a significantly greater severity of pain (P=0.004), bloating (P=0.013) and flatulence (P=0.006) during the fructan challenge compared to glucose, but not for any of the other challenges (Figure 3.6).

In addition, on the final day of the challenges, there were significant differences in the severity of pain (P=0.006), bloating (P<0.001), flatulence (P<0.001), borborygmi (P=0.036), faecal urgency (P=0.011), incomplete evacuation (P=0.026) and overall symptoms (P=0.006) across the challenges (Table 3.7). Following post hoc correction, there was a significantly greater severity of pain (P=0.004), bloating (P=0.002), flatulence (P=0.004) and faecal urgency (P=0.014) on the final day of the fructan challenge compared to glucose, but not for any of the other challenges.
Figure 3.6 Severity of gastrointestinal symptoms as measured by the GSRS during the challenges (0=absent, 1=mild, 2=moderate, 3=severe). Symptom severity was measured on each challenge day and a 3-day average was calculated. * P < 0.05 for the fructan challenge compared to glucose (placebo) challenge

The average severity of all 12 GI symptoms measured using the GSRS (absent=0, mild=1, moderate=2, severe=3) across the three challenge days was summed and divided by 36 (12 symptoms measured across three days) to obtain an average composite symptom severity score for each challenge. There was a significant difference in the composite score across the challenges (P<0.001), with post hoc correction revealing a significantly greater composite score during the fructan challenge (0.64, SD 0.45) compared to glucose (placebo) (0.38, SD 0.33) (P=0.004). There was no significant difference in any of the individual scores or in the composite score during the GOS or sorbitol challenges compared to glucose (placebo).
Table 3.7 Incidence of moderate or severe GI symptoms and severity of GI symptoms on the final day of the challenges

<table>
<thead>
<tr>
<th></th>
<th>Incidence (number of days) of moderate or severe symptoms during three-day challenge, mean (SD)</th>
<th>Severity of symptoms on the final day of challenges, mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fructan</td>
<td>GOS</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>0.9 (1.1)*</td>
<td>0.4 (0.8)</td>
</tr>
<tr>
<td>Bloating</td>
<td>1.0 (1.0)*</td>
<td>0.2 (0.6)</td>
</tr>
<tr>
<td>Flatulence</td>
<td>1.2 (1.2)*</td>
<td>0.6 (1.0)</td>
</tr>
<tr>
<td>Belching</td>
<td>0.3 (0.7)</td>
<td>0.1 (0.4)</td>
</tr>
<tr>
<td>Borborygmi</td>
<td>0.3 (0.9)</td>
<td>0.2 (0.6)</td>
</tr>
<tr>
<td>Faecal urgency</td>
<td>0.7 (1.1)</td>
<td>0.4 (0.9)</td>
</tr>
<tr>
<td>Incomplete evacuation</td>
<td>0.4 (0.9)</td>
<td>0.1 (0.3)</td>
</tr>
<tr>
<td>Nausea</td>
<td>0.1 (0.4)</td>
<td>0.0 (0.2)</td>
</tr>
<tr>
<td>Heartburn</td>
<td>0.1 (0.4)</td>
<td>0.0 (0.2)</td>
</tr>
<tr>
<td>Acid regurgitation</td>
<td>0.0 (0.2)</td>
<td>0.0 (0.2)</td>
</tr>
<tr>
<td>Lethargy</td>
<td>0.6 (1.1)</td>
<td>0.3 (0.8)</td>
</tr>
<tr>
<td>Overall</td>
<td>0.8 (1.1)</td>
<td>0.3 (0.6)</td>
</tr>
</tbody>
</table>

*a* Repeated-measures ANOVA across the challenges; *b* Severity scoring system: 0 = absent, 1 = mild, 2 = moderate, 3 = severe

* Significantly different from placebo (glucose) on Wilcoxon Signed Rank test with Bonferroni correction for multiple comparisons
3.5.3 Stool output

Stool frequency and consistency were assessed using the BSFS on each day of the challenges (Table 3.8). There was a significant difference in stool frequency during the challenges when compared across the challenges (P=0.026), although following post hoc correction this did not reach significance between any of the fermentable carbohydrate challenges alone compared to glucose (placebo) (Table 3.8). There was no significant difference in stool frequency on the final day of each challenge when compared across the challenges (P=0.412).

There was a significant difference in stool consistency across the challenges (p=0.010) and post hoc correction revealed softer stools (a higher score on BSFS) during the fructan (P=0.007) and GOS (P=0.033) challenges compared to glucose (Table 3.8). There was also a significant difference in stool consistency of the final day of the challenge across the challenges (P=0.017), although following post hoc correction this did not reach significance between any of the fermentable carbohydrate challenges alone compared to glucose.

Across the challenges, there was no significant difference in the proportion of stools that were of normal consistency (BSFS type 3, 4 or 5) (P=0.273), hard consistency (BSFS type 1 or 2) (P=0.051), or loose consistency (BSFS type 6 or 7) (P=0.269) during the challenges (Figure 3.7). There was no significant difference in the proportion of stools that were of normal consistency on the final day of the challenges (P=0.141).
Table 3.8 Stool frequency and consistency during and on the final day of the challenges

<table>
<thead>
<tr>
<th></th>
<th>Fructan</th>
<th>GOS</th>
<th>Sorbitol</th>
<th>Glucose</th>
<th>P value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>P value challenge vs. glucose&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fructan</td>
</tr>
<tr>
<td>Stool frequency (per day)</td>
<td>2.4 (1.4)</td>
<td>2.1 (1.2)</td>
<td>2.1 (1.5)</td>
<td>1.9 (1.2)</td>
<td><strong>0.026</strong></td>
<td><strong>0.067</strong></td>
</tr>
<tr>
<td>Stool frequency (final day of challenge)</td>
<td>2.3 (1.8)</td>
<td>2.1 (1.4)</td>
<td>2.1 (1.6)</td>
<td>1.9 (1.3)</td>
<td>0.412</td>
<td>-</td>
</tr>
<tr>
<td>Stool consistency (BSFS) during challenges&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.2 (1.3)</td>
<td>4.0 (1.0)</td>
<td>3.8 (1.2)</td>
<td>3.5 (1.0)</td>
<td><strong>0.010</strong></td>
<td><strong>0.007</strong></td>
</tr>
<tr>
<td>Stool consistency during challenges</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (type 3, 4 or 5)</td>
<td>71.0 (33.5)</td>
<td>78.5 (30.0)</td>
<td>74.3 (34.5)</td>
<td>66.6 (32.6)</td>
<td>0.273</td>
<td>-</td>
</tr>
<tr>
<td>Hard (type 1 or 2)</td>
<td>11.8 (26.5)</td>
<td>12.1 (24.3)</td>
<td>15.3 (31.5)</td>
<td>24.7 (29.5)</td>
<td>0.051</td>
<td>-</td>
</tr>
<tr>
<td>Loose (type 6 or 7)</td>
<td>17.2 (28.2)</td>
<td>9.4 (18.0)</td>
<td>10.4 (23.0)</td>
<td>8.7 (19.0)</td>
<td>0.269</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup>Repeated-measures ANOVA across the challenges; <sup>b</sup>Each challenge compared with placebo (glucose) using Wilcoxon Signed Rank test with Bonferroni correction for multiple comparisons; <sup>c</sup>Hard stools, BSFS type 1 or 2; normal stools, BSFS type 3, 4 or 5; loose stools, BSFS type 6 or 7
3.5.4 Biomarkers

Start and end of trial CRP concentrations were available for 27 participants (10 CD, 17 UC), and faecal calprotectin concentrations for 24 participants (10 CD, 14 UC) (Table 3.9). There was no significant difference in CRP between the start and end of trial (P=0.797). However, faecal calprotectin increased significantly between baseline (29.5 µg/g, SD 31.0) and end of trial (72.9 µg/g, SD 82.1) (P=0.018). However, only two participants (8%) had a faecal calprotectin >250 µg/g at end of trial, and the difference in end of trial faecal calprotectin between baseline and end of trial appears to be driven by large increases in five individuals (Figure 3.8). When sub-group analyses were performed individually for CD and UC, the difference in faecal calprotectin was significant only for CD between baseline (16.8 µg/g, SD 5.7) and end of trial (46.4 µg/g, SD 32.8) (P=0.026).

Table 3.9 Biomarkers at baseline (prior to challenge phase) and end of trial (after last washout period)

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD) Baseline</th>
<th>End of trial</th>
<th>P value a</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP (mg/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All participants (n=27)</td>
<td>2.3 (1.8)</td>
<td>2.4 (1.7)</td>
<td>0.797</td>
</tr>
<tr>
<td>CD (n=10)</td>
<td>2.1 (1.3)</td>
<td>2.6 (2.2)</td>
<td>0.487</td>
</tr>
<tr>
<td>UC (n=17)</td>
<td>2.5 (2.0)</td>
<td>2.3 (1.3)</td>
<td>0.624</td>
</tr>
<tr>
<td>Faecal calprotectin (µg/g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All participants (n=24)</td>
<td>29.5 (31.0)</td>
<td>72.9 (82.1)</td>
<td>0.018</td>
</tr>
<tr>
<td>CD (n=10)</td>
<td>16.8 (5.7)</td>
<td>46.4 (32.8)</td>
<td>0.026</td>
</tr>
<tr>
<td>UC (n=14)</td>
<td>38.6 (38.3)</td>
<td>91.9 (101.3)</td>
<td>0.083</td>
</tr>
</tbody>
</table>

Figure 3.7 Proportion of hard (BSFS type 1 or 2), normal (BSFS type 3, 4, or 5) and loose (BSFS type 6 or 7) stools during the challenges
Figure 3.8 Baseline and end of trial faecal calprotectin concentrations
3.6 Discussion

3.6.1 Gastrointestinal symptoms

The aim of this chapter was to report the results of a randomised, double-blinded, placebo-controlled, crossover, re-challenge trial designed to determine whether individual fermentable carbohydrates exacerbate FGS in patients with IBD in remission. The null hypothesis that fermentable carbohydrate challenges do not exacerbate FGS can be rejected since there was a significant difference in the proportion of participants reporting adequate relief on the final day (the primary outcome) and in individual GI symptom incidence and severity across the challenges compared to the placebo. This was particularly the case when analysed for the fructan challenge compared to placebo.

Fewer participants reported adequate relief on the final day of the fructan challenge compared to the placebo (glucose), reflecting a worsening of GI symptoms. This was supported by increased incidence and severity of abdominal pain, bloating and flatulence and is consistent with the proposed mechanisms of fermentable carbohydrates in FGS exacerbation. Fructans are a heterogeneous group of linear or branched fructose oligosaccharides and undergo minimal digestion in the human small intestine due to a lack of the $\beta$-fructofuranosidase enzyme required to hydrolyse the $\beta$-(2-1) fructosyl-fructose glycosidic bonds. Studies using ileostomy models and aspiration of distal ileal contents demonstrate that 87-89% of ingested fructans are recovered from the small intestine (Knudsen and Hessov, 1995, Molis et al., 1996, Ellegard et al., 1997), indicating that the majority are available for colonic bacterial fermentation with associated production of hydrogen, carbon dioxide and methane.

The mechanisms of fermentable carbohydrates in FGS induction or exacerbation have been examined in studies using functional MRI. A blinded fructan challenge compared to a placebo (glucose) significantly increased colonic gas volume in healthy volunteers (Murray et al., 2014, Major et al., 2017) and patients with IBS (Major et al., 2017). However, GI symptoms were significantly exacerbated only in the patients with IBS but not in healthy volunteers. Therefore, symptom exacerbation following the fructan challenge observed in this trial is supported mechanistically.

Although significantly fewer patients reported adequate relief of FGS on the final day of the fructan challenge compared to placebo (glucose), there was no significant differences in the proportion of participants reporting adequate relief on all three days of the challenges or in the mean number of days of adequate relief during the challenges. One possible explanation for this discrepancy is a cumulative effect of fermentable carbohydrates, with FGS becoming progressively worse with prolonged intake. The lack of symptom exacerbation when analysing
all three challenge days, but a significant difference when analysing just the final day of the challenge may indicate a symptom peak on the final day of the fructan challenge compared to the first two days of the challenge. A cumulative effect of on-going fermentable carbohydrate intake on GI symptoms is yet to be established in other studies.

Although fructans at a dose of 12 g/d induced GI symptoms compared to placebo (glucose), the same effects were not observed during the GOS or sorbitol challenges (6 g/d). A soybean-derived α-GOS (including raffinose, stachyose and verbascose) was chosen in this study to mimic GOS found in the diet (e.g. pulses, grains and nuts). Mammals lack the α-galactosidase enzyme required for monosaccharide liberation by hydrolysis of α-1,6 linkages within α-GOS, therefore dietary α-GOS is assumed to reach the colon intact and become susceptible to bacterial fermentation. Mechanistic studies investigating the digestion of α-GOS in humans are limited to raffinose, with 88% passing through the small intestine undigested in a small study of patients with ileostomies (Saunders and Wiggins, 1981).

The gas-forming capacity of legumes has been known for some time, with several studies in healthy human volunteers establishing increased flatulence following consumption of different types of legumes (Steggerda et al., 1966, Calloway et al., 1971, Suarez et al., 1999). Although these studies were not conducted in patients with IBD or IBS, they indicate that α-GOS-containing foods increase colonic gas production and therefore have the potential to exacerbate FGS through luminal distension. The gas-forming properties of legumes relate to colonic bacterial fermentation of α-GOS contained within them (Singh et al., 2017), supported by an animal study demonstrating greater gas production following α-GOS-containing soy milk compared to α-GOS-depleted soy milk (LeBlanc et al., 2008).

Interestingly, the case-control study described in Chapter 2 revealed significantly lower GOS intake in inactive IBD with FGS compared to healthy controls. This indicated a possible relationship between GOS and GI symptoms in inactive IBD, which seems discordant with the lack of GI symptoms following GOS challenge in this trial. However, the effect of GOS in food form may differ to that in pure form.

The lack of symptom exacerbation observed during the GOS challenge could be explained in several ways. Firstly, it could be that α-GOS do not induce FGS in patients with IBD. This could relate to the metabolism and interactions of the complex bacterial community in the GI tract, which is known to be altered in patients with IBD compared to healthy controls (Sartor and Mazmanian, 2012). Hydrogen produced via fermentation of dietary α-GOS may be consumed by other microbes. Methanogens (e.g. Methanobrevibacter smithii) and sulphate-reducing bacteria (e.g. Desulfovibrio spp.) consume hydrogen and carbon dioxide, reducing the gas molar volume.
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of the GI lumen (Blaut, 1994, Gibson et al., 1993). Therefore, individuals harbouring high numbers of these species may experience fewer symptoms attributable to colonic gas accumulation (e.g. bloating, flatulence) upon consumption of fermentable carbohydrates. If this were the case, however, fructans would be expected to be similarly tolerated in these individuals, which given the significant increase in symptoms following the fructan challenge, was not the case.

Secondly, the daily dose of GOS (6 g/d) may have been insufficient to significantly exacerbate FGS, compared to the fructan dose (12 g/d). The doses were chosen to reflect the highest achievable daily intake of GOS in the UK, based on limited dietary intake data that suggested an average GOS intake of 2 g/d in patients with IBS (Staudacher et al., 2012). In contrast, fructan intakes in the UK are greater than GOS, at 3.6-4.0 g/d in healthy individuals (Table 3.2), patients with IBS and patients with inactive CD (Dunn et al., 2011, Staudacher et al., 2012, Anderson et al., 2015). It was therefore deemed inappropriate to administer equal doses of fructans and GOS in the current study. Regardless of the different doses of GOS and fructans used in the current study, the GOS dose was high compared to the average GOS intake of patients with IBS in the UK (2 g/d), and therefore the lack of significant symptom induction during this challenge is surprising.

Thirdly, different varieties of beans and pulses contain variable quantities and combinations of α-GOS and these are altered to differing degrees during processing methods such as soaking and boiling (Brummer et al., 2015). Different species of pulses have been identified as containing variable proportions of raffinose, stachyose and verbascose (Guillon and Champ, 2002). It is therefore likely that α-GOS from different pulse varieties are fermented to differing degrees by different bacteria and the use of α-GOS derived from another variety in this study may have rendered different effects on FGS. Additionally, other carbohydrate fractions of pulses such as resistant starch are fermented by certain GI bacteria and therefore their effects on FGS in IBD should be investigated (Yang et al., 2013a).

Sorbitol is a polyol found naturally within various fruits and vegetables (Yao et al., 2014) and, owing to poor intestinal absorption and reduced caloric value compared to sugar, is frequently added to low calorie products (Zumbe et al., 2001). Mannitol, another common polyol, significantly increased small bowel water content in healthy volunteers when given as a pure carbohydrate challenge of 17.5 g (Marciani et al., 2010). Osmotic effects upon ingestion of polyols likely result from their incomplete intestinal absorption, which has been demonstrated in an ileostomy study (Langkilde et al., 1994). Complete absorption of a 10 g pure sorbitol challenge, determined using breath hydrogen concentration following the challenge, was observed in 40% of patients with IBS and 33% of healthy volunteers (Yao et al., 2014). In that
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study, GI symptoms significantly increased following the sorbitol challenge in patients with IBS, although malabsorption of the polyols was not required for symptom generation. Other studies have demonstrated increased GI symptoms following a 10 g dose of sorbitol in > 30% of healthy subjects (Corazza et al., 1988, Jain et al., 1987).

The lack of symptom induction following the sorbitol challenge in the current study, in contrast to the aforementioned studies, may relate to differences in the dose of polyol administered (10 g in previous studies vs. 6 g in the current study). Furthermore, patients in the aforementioned studies took the polyol challenges after an 8-hour fast, in contrast to the current study in which patients were permitted to take the challenge drink alongside normal diet. Gastric emptying of liquid sorbitol was slower when ingested with a solid meal than when taken alone in healthy volunteers (Skoog et al., 2006), therefore sorbitol may reach the small intestine more rapidly under fasted conditions and thus induce more noticeable luminal distension and symptom exacerbation.

Finally, it has been demonstrated that doses of sorbitol much in excess of those used in the current study (27-30 g) can be well tolerated in healthy volunteers, with just an increase in flatulence compared to glucose (Skoog et al., 2006, Beaugerie et al., 1990). Although these studies were conducted in people without GI symptoms, they again indicate that the lack of symptom exacerbation following the sorbitol challenge in the current study may be explained by the relatively small dose used.

It could be argued that since the fructan dose (12 g/d) was double that of the GOS and sorbitol doses (6 g/d), the symptom exacerbation following fructans may simply reflect a greater load of fermentable carbohydrate and may not be a fructan-specific effect. Although there was sound rationale for using different doses of different FODMAPs, further research would be required to elucidate whether GOS and sorbitol in doses of 12 g induce FGS in patients with IBD.

Despite the seemingly high fructan dose of 12 g/d used in this study, a total habitual FODMAP intake of 29.6 g/d has been observed in patients with IBS in the UK, with low FODMAP dietary advice resulting in an 11.9 g/d reduction in total FODMAP intake (Staudacher et al., 2012). Therefore the 12 g daily dose of fructans likely still represents a relatively typical dietary FODMAP intake. Furthermore, the only previous study of this nature administered 19 g/d of fructans, a larger dose than that used in the current study (Shepherd et al., 2008).

3.6.2 Stool output

During the fructan and GOS challenges there was significantly softer stool consistency compared to placebo. There were no significant differences in stool frequency or the proportion of stools
classified as normal, loose or hard during any of the fermentable carbohydrate challenges compared to placebo.

Fructans and GOS are types of dietary fibre (Flamm et al., 2001). Fibre is generally accepted to accelerate whole-gut transit time through bulking of luminal contents (Brownlee, 2011, Chaplin, 2003). Although fructans and GOS are rapidly and completely fermented by the colonic microbiota and thus do not have a high water-binding capacity, these highly fermentable fibres may influence stool frequency and consistency through increased biomass, short-chain fatty acid production and reduced pH (Blackwood et al., 2000, Flamm et al., 2001).

Fructan supplementation significantly reduced defaecation difficulties in elderly patients (Marteau et al., 2011), a finding that has been replicated for GOS (Surakka et al., 2009). Furthermore, a recent randomised, placebo-controlled trial in children demonstrated a softening of stool form following inulin supplementation compared to placebo (Closa-Monasterolo et al., 2016), and chicory inulin supplementation increased stool frequency and reduced the proportion of participants with hard stools in adults with constipation (Micka et al., 2017).

These previous studies demonstrate the capacity of fructans to influence stool form, as observed in the current study. However, the above studies were conducted in patients with constipation, in contrast to the current study in which patients met the criteria for either IBS, functional bloating or functional diarrhoea. Additionally, some studies have demonstrated no effect of inulin on stool frequency or consistency in healthy volunteers and patients with constipation (Slavin and Feirtag, 2011) (Linetzky Waitzberg et al., 2012). Furthermore, a meta-analysis of fibre supplementation in patients with chronic constipation showed that psyllium, a soluble fibre that is slowly and partially fermented (Kaur et al., 2011), exerted a greater effect on stool frequency and consistency than inulin and GOS (Christodoulides et al., 2016). However, unsurprisingly, inulin and GOS did increase faecal Bifidobacteria counts.

Interestingly, no significant differences in stool frequency or consistency were observed during the sorbitol challenge compared to placebo. Sorbitol has long been thought to have laxative effects (Peters and Lock, 1958), owing to an increase in luminal water resulting from incomplete absorption and low molecular weight (Yao et al., 2014). Sorbitol is therefore used under certain circumstances for the relief of chronic constipation (Lederle et al., 1990), although there is a paucity of RCT evidence to support this (Ramkumar and Rao, 2005). A study in healthy volunteers demonstrated accelerated colonic transit, but no effect on stool frequency or consistency, following a 27g dose of sorbitol (Skoog et al., 2006). Although this study was not performed in patients with IBD or IBS, it demonstrates that doses of sorbitol more than 4 times
that used in the current study can be tolerated by some individuals. The dose of sorbitol used in
the current study therefore may be insufficient, or a longer period of consumption may be
required, to exert effects on stool output.

3.6.3 Biomarkers

Faecal calprotectin increased significantly between baseline (prior to randomisation) and end of
trial, and sub-group analyses of CD and UC revealed that this effect was specifically observed in
patients with CD only. In any IBD trial, there lies the potential for a natural fluctuation in disease
activity, irrespective of the intervention under investigation. A significant increase in FCP during
the trial may not necessarily indicate an increase in disease activity in patients with CD, but may
represent subtle changes in intestinal inflammation.

While it has been demonstrated that faecal calprotectin concentration in patients with active
UC is reproducible in different aliquots of the same stool sample, it may fluctuate significantly
in different stools passed over a single day and over two consecutive days in the same individual
and faecal calprotectin concentrations increased with length of time between bowel
movements (Lasson et al., 2015). Conversely, faecal calprotectin concentrations on three
consecutive days were similar in patients with inactive CD, suggesting that faecal calprotectin
may be more stable in inactive IBD (Naismith et al., 2013). Whether the rise in faecal calprotectin
observed in this study in an individual sample in patients with CD represents an increase in GI
inflammation or a natural fluctuation is unclear.

A prebiotic is defined as a substrate that is selectively utilised by host microorganisms conferring
a health benefit (Gibson et al., 2017). Numerous studies have demonstrated that fructans and
GOS selectively stimulate Bifidobacteria and Lactobacilli in healthy human subjects (Roberfroid
et al., 2010). The distinction should be made between α-GOS, derived from beans and pulses
(e.g. raffinose, stachyose, verbascose), and β-GOS, which have a terminal β-linked glucose unit
and are commercially synthesised from lactose. These differing types of GOS likely have different
prebiotic potential (Vulevic et al., 2004, Bouhnik et al., 2004) and the majority of studies
assessing the prebiotic activity of GOS to date have focussed on β-GOS.

The GI microbiota can interact directly (through interaction of microbial associated molecular
patterns with pattern recognition receptors on immune cells) and indirectly (via, for example,
bacterial metabolites such as butyrate) with the host immune system (Kamada and Kao, 2013).
Certain GI bacteria, including Bifidobacteria, Lactobacilli and Faecalibacterium prausnitzii,
exhibit anti-inflammatory or immune-modulatory effects in vitro and in vivo (Breyner et al.,
2017, Fanning et al., 2012, Kelly et al., 2005). The GI microbiota are altered in IBD compared to
healthy individuals, most notably characterised by a reduction in the abundance of F. prausnitzii,
particularly in CD (Pascal et al., 2017). Furthermore, 15 g/d fructan supplementation in patients with active CD significantly reduced the proportion of mucosal IL-6 positive dendritic cells (DC) and increased IL-10 staining in DC, indicating that fructans may influence mucosal immune function in CD (Benjamin et al., 2011b). This occurred in the absence of alterations to Bifidobacteria or *F. prausnitzii*, indicating potentially direct effects of prebiotics on the GI immune system.

The low FODMAP diet requires a restricted intake of dietary prebiotic carbohydrates (fructans and α-GOS). Unsurprisingly, several RCTs have demonstrated alterations to the gut microbiota following the diet (Staudacher et al., 2012, Halmos et al., 2014a, McIntosh et al., 2016), including reduced Bifidobacteria and *F. prausnitzii*. It is possible that these changes could impact upon host immune function, although the length of FODMAP restriction required for immunological changes to occur is yet to be elucidated. In this trial, participants followed a strict low FODMAP diet prior to and during the re-challenge trial. Whether the rise in faecal calprotectin observed between the start and end of the trial is related to GI microbiota changes related to dietary prebiotic restriction is unclear, given the lack of GI microbiota assessment in this trial. This issue is further complicated by the pure FODMAP challenges that overlaid the FODMAP restriction, which could be seen as a replacement of the restricted dietary prebiotic. Further research is required to clarify the effect of dietary fermentable carbohydrate restriction and challenge on biomarkers of intestinal inflammation in IBD.

### 3.6.4 Significance of results

This is the first trial to establish that fermentable carbohydrates exacerbate GI symptoms in patients with inactive IBD. The symptom exacerbation observed following the fructan challenge is supported mechanistically and demonstrates the ability of FODMAPs to exacerbate FGS in inactive IBD.

The lack of symptom exacerbation following the GOS and sorbitol challenges, in doses at least three times the average dietary UK intake, could be interpreted to suggest that the low FODMAP diet, as implemented to date, is excessively restrictive. It could be argued that if patients can tolerate doses of GOS and sorbitol much in excess of average intake, the diet could be made more flexible and thus more acceptable to patients. However, caution should be applied in extrapolating the results of a proof-of-concept study of this nature directly to clinical practice. Participants were given GOS and sorbitol in pure form and were permitted to take them with or without food. The physiological responses to the carbohydrate challenges may differ to those taken within foods. Additionally, the effects of different doses of GOS and sorbitol require further investigation in the context of FGS in inactive IBD. Although this study does not
demonstrate efficacy of the low FODMAP diet in this patient group, it suggests that a randomised, placebo-controlled trial of the low FODMAP diet in IBD in remission is warranted.

3.6.5 Strengths and limitations

This is the first randomised, placebo-controlled, double-blinded crossover re-challenge trial to investigate the effects of fermentable carbohydrate challenges on FGS in patients with inactive IBD. The study was designed to identify whether fermentable carbohydrates exacerbate FGS in IBD while overcoming the inherent methodological difficulties associated with establishing adverse food reactions through restriction of dietary components, namely the potentially large placebo responses and the possibility of altering dietary components other than those under investigation.

It was vital that this trial included a placebo challenge, since significant symptom responses to placebo challenges such as potato starch, sucrose and glucose have been observed in food elimination and re-challenge studies (Yao et al., 2013). Around 10% of participants reported that they did not have adequate relief following the glucose challenge, which is comparable to 14% of patients with IBS in the only other trial of this nature (Shepherd et al., 2008).

The observed effect size of the fructan challenge in the current trial was lower than predicted from the previous IBS trial (Shepherd et al., 2008). It was expected that 50% of participants would report inadequate relief of GI symptoms following the fructan challenge, compared to 14% during the glucose placebo (section 3.4.12). However, 38% of participants reported inadequate relief following the fructan challenge compared to 10% following the glucose. A post hoc sample size estimation indicates that 38 participants would be required to detect a difference between groups based upon these findings, suggesting that the trial may have been under-powered to detect differences between the groups with this smaller effect size.

Open challenges have been demonstrated to be of little value in establishing adverse food reactions due to the large ‘nocebo’ response, as described above (Yao et al., 2013). Maximal blinding of participants and researchers reduces ascertainment bias and is particularly important in FBD trials (Irvine et al., 2016). Although participants in this trial were aware that the objective was to assess the effect of fermentable carbohydrates on GI symptoms, they were not informed of the carbohydrates under study and therefore remained blinded. Although the fructan and glucose challenge were double the dose of the GOS and sorbitol challenges and would have appeared to be a larger quantity of powder, this did not appear to increase the symptom responses since around 90% of participants reported adequate relief following the glucose challenge. Researchers involved in trial recruitment were blinded to the order of carbohydrate challenges received by each participant.
Crossover study designs have been frequently used in functional bowel disorder trials (AKEHURST and KALTENTHALER, 2001). The overarching advantage of this trial design is the possibility of a smaller sample size, since each individual serves as their own control (Miller, 2014). This is particularly relevant to trials with several treatment arms, where parallel trials may become infeasibly large. Despite this, the possibility of carryover effects has cast debate over the suitability of crossover trials for FBDs. Nonetheless, a crossover design was judged most appropriate for this trial since there were four treatment arms, the challenges were of short duration, and it was anticipated that the physiological effects of the challenges (GI symptoms) would be short-lived and therefore unlikely to continue into the following challenge (Miller, 2014). Furthermore, participants were not permitted to commence a challenge until adequate relief of GI symptoms was achieved, thus reducing the chance of carryover effects.

Adherence to the low FODMAP diet during the trial was crucial in order to conclude with confidence that the challenges were responsible for any symptom responses observed. Currently, a validated tool for measuring compliance to this dietary intervention does not exist. Although participants reported good adherence (defined as $\geq 75\%$ adherence on all three challenge days) to the low FODMAP diet, dietary intake was not recorded in a food diary, and therefore it is impossible to assess for accidental or deliberate FODMAP exposure. Subjective ratings of compliance are prone to error, since participants may report the level of compliance that they perceive to be desirable to the researcher. However, completing food diaries can be burdensome and as a result can alter dietary intake (Lewis et al., 2017). In this trial, dietary intake would need to be recorded for at least 12 days ($4 \times 3$-day challenges). It was felt that this would compromise participant retention in the trial, and that the accuracy of recording could decline as the participant fatigues.

The doses of the carbohydrate challenges were chosen based on previous provocation studies and upon typical dietary intakes of the carbohydrates. Since typical UK intakes of fructans exceed those of GOS and sorbitol, a higher dose was chosen for this carbohydrate (12 g/d vs. 6 g/d). This could be viewed as both a strength and limitation of this trial. Providing doses that reflect the higher end of typical intake potentially makes the findings more clinically relevant, demonstrating the effects of the carbohydrates as consumed within a normal diet. Providing 12 g/d of all of the challenges would be equal to providing c. 1kg chickpeas for GOS or eight plums for sorbitol (Muir et al., 2007, Muir et al., 2009). On the other hand, challenging with different doses of carbohydrates renders the findings difficult to interpret; whether the increase in GI symptoms observed during the fructan challenge is specific to fructans or simply due to the greater dose administered requires clarification in future studies.
3.7 Conclusion

This trial established that a pure fructan challenge exacerbates FGS in patients with IBD in remission following FGS control with a low FODMAP diet. This may appear to suggest the need for dietary fructan restriction for the control of FGS in IBD remission, as opposed to a broader fermentable carbohydrate restriction (the low FODMAP diet). However, the limitations associated with this trial (namely, the varying doses of fermentable carbohydrates used) and the difficulties in extrapolating the results to clinical efficacy of dietary carbohydrate restriction are such that the results of this trial can only be interpreted as an indication that fermentable carbohydrate restriction diet may be of benefit in IBD.

A recent trial demonstrated that compared to patients with IBD in remission following their habitual diet, patients randomised to the low FODMAP diet experienced an improvement in FGS and health-related quality of life (Pedersen et al., 2017). This, together with the evidence from the current re-challenge trial that fermentable carbohydrates may have a role in FGS exacerbation in IBD, confirms the need for a randomised, placebo-controlled trial of the low FODMAP diet in IBD in remission.
Design and methods of a randomised controlled trial to investigate the effect of fermentable carbohydrate restriction on functional gastrointestinal symptoms, gut microbiota and blood immunology in patients with inactive IBD
4.1 Introduction

The evidence that dietary fermentable carbohydrate restriction in IBD is effective is growing. Uncontrolled studies have suggested an improvement of GI symptoms in patients with inactive IBD following the low FODMAP diet in clinical practice (Gearry et al., 2009a, Prince et al., 2016). Furthermore, compared to patients following their habitual diet, the low FODMAP diet resulted in improved GI symptom severity in a randomised controlled trial (RCT) of patients with inactive or mildly to moderately active IBD (Pedersen et al., 2017). This was the first RCT of the low FODMAP diet in IBD, however this trial was not placebo-controlled.

Studies of dietary advice for GI symptoms are fraught with issues including the choice of control treatment. Allocating the control group to a habitual diet renders the trial unblinded and unable to control for the potentially substantial placebo response in functional bowel disorders such as IBS (Elsenbruch and Enck, 2015), while the gold standard placebo ‘sham’ diet is extremely challenging to design. Comparing the treatment of interest to a standard treatment allows superiority or inferiority of the new treatment to be established, however both treatments may produce a placebo response which is unaccounted for. Furthermore, blinding can be difficult since the standard treatment may be commonly known. Another issue faced by researchers investigating dietary interventions is that posed by the complexity of whole foods, with the possible inadvertent manipulation of dietary components that may influence the study outcomes independent of the component under investigation (Yao et al., 2013). The lack of a placebo control diet in the aforementioned trial of the low FODMAP diet in functional gastrointestinal symptoms (FGS) in inactive IBD (Pedersen et al., 2017) is a major limitation given the substantial placebo responses that have been observed in both IBS and IBD (Elsenbruch and Enck, 2015, Su et al., 2004, Jairath et al., 2016). Detailed dietary advice provided by a healthcare professional can induce a powerful placebo response and comparing to a control group not provided with any advice results in uncertainty around the degree of placebo response induced.

This study also lacked an assessment of the GI microbiota before and after the diets, and several previous trials in IBS have revealed a profound effect of the low FODMAP diet on the composition of the GI microbiota (Staudacher et al., 2012, Staudacher et al., 2017b). This may be particularly relevant in patients with IBD given the divergence of the microbiota from that of healthy controls that likely already exists in a large proportion of patients (Halfvarson et al., 2017, Pascal et al., 2017). A reduction in immune-regulatory Bifidobacteria, such as has been observed in several studies of the low FODMAP diet in IBS, has the potential alter GI immune function (Sarkar and Mandal, 2016). Furthermore, the products of degradation of complex polysaccharides by Bifidobacteria (e.g. acetate) are substrates for other members of the GI
microbiota (Turroni et al., 2016). Such cross-feeding reactions mean that a depletion in Bifidobacteria has the potential to influence other beneficial bacteria, such as Faecalibacterium prausnitzii (Moens et al., 2016), which has in vitro and in vivo anti-inflammatory actions (Miquel et al., 2013). Therefore, through effects on the GI microbiota, the low FODMAP diet has the potential to impact upon the inflammatory process in IBD, although previous studies of the low FODMAP diet in IBD have failed to measure immunological sequelae.

Additionally, studies assessing the GI microbiota in IBS following a low FODMAP diet have used quantification methods such as fluorescence in situ hybridisation (FISH) (Staudacher et al., 2012) and quantitative polymerase chain reaction (qPCR) (Halmos et al., 2014a, Staudacher et al., 2017b), and two studies have used a 16S rRNA sequencing approach (McIntosh et al., 2016). The former methods (qPCR and FISH) allow quantification of only a selection of bacteria, while 16S rRNA sequencing characterises the composition of the GI microbiota but provides no information on the potential functions of the microbiota.

Therefore, the aim of this RCT was to address the aforementioned issues in design of low FODMAP diet trials and the lack of randomised, placebo-controlled evidence for the low FODMAP diet in IBD. The RCT was designed to investigate the effect of low FODMAP dietary advice compared to placebo sham dietary advice on GI symptoms, GI microbiota composition and function, blood immunology and inflammatory markers in patients with inactive IBD.

4.1.1 Aims of the RCT

The aim of this RCT was to investigate the effect of low FODMAP dietary advice compared to placebo ‘sham’ dietary advice on FGS, GI microbiota, circulating T-cell gut-homing phenotype and markers of intestinal inflammation in patients with inactive IBD with FGS.

Primary objective: to investigate the change in symptom severity score (IBS-SSS) following low FODMAP dietary advice compared to placebo 'sham' dietary advice in patients with inactive IBD with FGS.

Secondary objectives:

To investigate the effect of low FODMAP dietary advice compared to placebo ‘sham’ dietary advice on the following outcomes in patients with inactive IBD with FGS:

1. GI symptoms and stool output:
   a. Adequate relief of GI symptoms (global symptom question)
   b. Individual GI symptom incidence and severity (gastrointestinal symptom rating scale)
   c. Stool frequency and consistency (Bristol stool form scale)
2. Clinical disease activity (Harvey Bradshaw Index for CD and Partial Mayo Score for UC)
3. Patient perceived control of IBD (IBD control questionnaire)
4. Health-related quality of life (UK inflammatory bowel disease questionnaire, IBDQ)
5. Food-related quality of life (Food-related quality of life questionnaire)
6. Biomarkers of intestinal inflammation:
   a. Faecal calprotectin
   b. Serum C-reactive protein
7. GI luminal environment:
   a. Faecal microbiota composition and function
   b. Faecal SCFA concentration
   c. Faecal pH
   d. Faecal volatile organic compounds
8. Circulating (blood) T-cell phenotype and gut homing marker expression
9. Nutrient intake
10. Diet acceptability

4.1.2 Hypotheses

Null (H0) hypothesis:
There is no difference in the change in symptom severity score (IBS-SSS) at four weeks following low FODMAP dietary advice compared to placebo sham dietary advice in patients with inactive IBD with FGS.

Alternative hypothesis:
There is a difference in the change in symptom severity score (IBS-SSS) at four weeks following low FODMAP dietary advice compared to placebo sham dietary advice in patients with inactive IBD with FGS.

4.2 Interventions, trial design and approvals

4.2.1 Trial design

The major objectives of treatment trials for functional bowel disorders (FBD) are to ascertain whether the intervention of interest relieves symptoms, improves health-related quality of life, improves ability to cope with the symptoms and decreases the use of healthcare resources, while avoiding harm (Irvine et al., 2016). The randomised, double-blind, placebo-controlled parallel group trial reduces major sources of bias and confounding and therefore remains the gold standard method to investigate efficacy of a new treatment (Kramer and Shapiro, 1984, Irvine et al., 2016). Trials in FBD pose unique challenges including the high placebo response characteristic of these conditions (Elsenbruch and Enck, 2015), the lack of biomarkers and
subjectivity of symptom reporting, fluctuation of symptoms, heterogeneous symptom aetiology, difficulty in blinding participants and investigators in trials of behavioural interventions, and a lack of appropriate and validated endpoints for some types of FBD (Irvine et al., 2016).

Furthermore, the background of inactive IBD in the current trial may influence GI symptoms and introduces potential confounding factors including medications, prior dietary restrictions, prior GI surgery and heterogeneous IBD phenotype.

As outlined above, dietary intervention trials pose challenges not encountered in pharmacological treatment trials. One complexity of dietary trials is the choice of an appropriate control or comparator group, since designing placebo dietary advice is extremely challenging. Blinding participants and investigators to the treatment allocation is also problematic compared with drug trials, since a dietary intervention is clearly easier to identify than a pill (Yao et al., 2013). Finally, with no recognised reporting guidelines for dietary intervention trials, the quality of trials and papers reporting dietary interventions is highly variable.

This study was a multi-centre, single-blinded, randomised, parallel placebo-controlled trial conducted in line with CONSORT recommendations. The trial protocol and procedures are illustrated in Figure 4.1. Although participant blinding was made possible through using placebo dietary advice, it was not possible to blind the investigator providing the advice since dietary counselling was required. Dietary counselling was used to mimic clinical practice (except for an explanation of the proposed mechanisms of the diet) and to maximise participant compliance to the dietary interventions.

The duration of the dietary intervention was four weeks, based upon previous studies of 3-4 weeks duration demonstrating efficacy of the low FODMAP diet in IBS (Staudacher et al., 2012, Halmos et al., 2014b, McIntosh et al., 2016, Eswaran et al., 2016, Bohn et al., 2015, Staudacher et al., 2017b). Furthermore, although not all women have a 28-day menstrual cycle, a 4-week trial may reduce the potential confounding effect of menstruation on GI symptoms in a proportion of female subjects, which may be enhanced in patients with IBD, particularly CD (Bernstein et al., 2012), and in IBS (Heitkemper et al., 2003).
Chapter 4: RCT design and methods

Figure 4.1 The RCT protocol and procedures; PGA, Physician Global Assessment; CRP, C-reactive protein; FCP, faecal calprotectin; GI, gastrointestinal; QoL, quality of life; SCFA, short-chain fatty acids
4.2.1.2 Trial sites

Trial recruitment took place at Guy’s and St Thomas’ NHS Foundation Trust and Barts Health NHS Trust, London, UK. All participants were recruited by the thesis author from gastroenterology and dietetic outpatient clinics and trial visits took place in the hospital clinic or in the Department of Nutritional Sciences, King’s College London.

4.2.1.3 Patient selection

Patients were recruited between February 2016 and April 2017. Enrolled patients were those that met the following criteria:

4.2.1.3.1 Inclusion criteria

- Men and women aged ≥18 years
- Diagnosis of IBD, as defined by:
  - Standard clinical, histological and radiological criteria
  - Diagnosis at least 6 months prior to screening
- Inactive disease, as defined by:
  - Physician Global Assessment
  - Faecal calprotectin <250 μg/g
  - Serum CRP <10 mg/L
  - Stable medications (see exclusion criteria)
  - No GI surgery in the 6 months preceding screening
  - No IBD flare in the 6 months preceding screening
- GI symptoms fulfilling the following criteria:
  - Have been assessed with objective investigations and thought not to be a result of active inflammation, and which do not require escalation of IBD medications
  - Meet the Rome III criteria for IBS-D, IBS-M, IBS-U, functional bloating or functional diarrhoea
  - Meet the required threshold:
    - Lack of adequate relief of symptoms during the screening week
    - Mild, moderate or severe abdominal pain, bloating or diarrhoea on at least 2 days during the screening week
- Have not been exposed to a low FODMAP diet in the past
- Individuals able to give informed consent
- A willingness to participate
4.2.1.3.2 Exclusion criteria

- Any evidence of active disease, defined as:
  - Requiring escalation in medications as assessed by the treating physician
  - Currently taking steroids
  - GI surgery is thought to be imminent
- Changes in dose of azathioprine, 6-mercaptopurine, methotrexate or anti-TNF-α agents in the preceding 12 weeks, or oral 5-ASA in the preceding four weeks
- Constipation-predominant symptoms (either meeting criteria for IBS-C or functional constipation)
- Use of antibiotics, probiotics or prebiotics in the preceding eight weeks or non-steroidal anti-inflammatory drugs in the preceding week
- Pure perianal disease
- Current stoma
- Other factors likely to be the cause of gut symptoms:
  - Extensive colonic or small intestinal resection
  - Symptoms thought to relate to a current stricture
  - Taking any medications with the potential to influence GI symptoms, e.g. lactulose, loperamide or senna, unless taking long-term stable dose that is unlikely to change or stop during the trial
  - Individuals with established bile acid malabsorption
- Comorbidities:
  - Sepsis or fever
  - Diabetes or coeliac disease
  - Other concomitant serious comorbidity e.g. significant hepatic, renal, endocrine, respiratory, neurological or cardiovascular disease
- Seen by a dietitian in the preceding 6 months for advice regarding nutrition support
- Pregnancy or lactation
- Full bowel preparation for a diagnostic or monitoring procedure in preceding 4 weeks

4.2.1.4 Intervention

The principles and practical aspects of the low FODMAP diet are described in section 1.4. Briefly, this diet requires exclusion of foods high in fructans, galacto-oligosaccharides, lactose, fructose and polyols (including sorbitol and mannitol). This was achieved through provision of food lists to participants, which clearly stated foods that were permitted (low in fermentable carbohydrates) and foods that were to be avoided (high in fermentable carbohydrates).
As discussed in section 4.1, use of a placebo group is particularly desirable in trials of functional bowel disorders given the considerable placebo response in these conditions (Elsenbruch and Enck, 2015). Placebo responses of 10-33% have also been observed in maintenance and treatment trials in IBD (Jairath et al., 2016, Su et al., 2004). To overcome the lack of placebo dietary advice for use in comparison to the low FODMAP diet, placebo ‘sham’ dietary advice had been developed previously by our group (Staudacher et al., 2017b). Three previous trials have utilised sham dietary advice effectively in patients with CD, two of which investigated an exclusion diet based upon IgG-specific responses (Bentz et al., 2010, Gunasekera et al.) and one that investigated a low microparticle diet (Lomer et al., 2005).

The aims of the sham dietary advice were as follows:

1. To provoke dietary modifications in patients in the control group, while masking diet allocation, such that a placebo response may be generated
2. To maintain an intake of macronutrients and micronutrients comparable to baseline
3. To maintain an intake of fermentable carbohydrates comparable to baseline

Development of the sham diet began with a review of suitable and unsuitable foods on the low FODMAP diet. Foods likely to contribute significantly to UK FODMAP intake (e.g. wheat, onion, garlic, apples, pears) were allocated to the ‘suitable’ foods list, since restricting these would likely restrict overall FODMAP intake. Meanwhile, foods less likely to make a significant contribution to FODMAP intake, such as rye and millet, were allocated to the ‘restricted’ list. There was also consideration of foods that, when restricted, may influence intake of other foods that are typically eaten alongside them. For example, tomatoes are often eaten in dishes containing onion and garlic, and therefore tomatoes were allocated to the ‘suitable’ food list.

Certain principles of the low FODMAP diet were consistent within the sham diet; for example, while meat and meat products were generally on the ‘suitable’ list, those containing unsuitable ingredients, such as chives, were unsuitable. The sham diet was shown to be successful in a large RCT of patients with IBS, where nutrient and FODMAP intake were not changed by the diet (Staudacher et al., 2017b)

All patients randomised to the trial (in both groups) completed a 7-day food diary prior to randomisation, which was assessed at the baseline visit. The relevant dietary advice was then provided, consisting of counselling on the foods permitted and excluded without an explanation of the proposed mechanisms of the diet, a process which took approximately 20 minutes for all participants. Written dietary resources were provided to all participants.
4.2.1.5 Sample size calculation

The sample size calculation was based on the primary endpoint, the change in symptom severity score (IBS-SSS) between baseline and end of trial in the low FODMAP diet compared to the placebo sham diet groups.

The estimate of the effect size of the low FODMAP diet was based on the findings of an RCT assessing the effects of a low FODMAP diet compared to a placebo sham diet in patients with IBS (Staudacher et al., 2017b). Design of the current trial was similar in its use of the same placebo sham diet although the patient groups were different (IBD vs. IBS). However, in the absence of any published RCTs of the low FODMAP diet in IBD at the time of calculating the sample size, this trial was the most similar on which to base the sample size. Furthermore, the aim of the current RCT was to examine the effect of the low FODMAP diet in patients meeting the criteria for functional bowel disorders with a background of IBD, therefore it could be expected that the effect size would be similar to that in patients with IBS.

In the previous trial, the mean change in IBS-SSS score between baseline and week four in the low FODMAP diet group and sham diet group was -118 (SD 86) and -44 (SD 72), respectively. The sample size calculation was performed by hand using a formula for the comparison of two sample means (Bland, 2000):

\[
n = \frac{f(\alpha, P) \sigma^2 \cdot 2}{(\mu_1 - \mu_2)^2}
\]

\[
n = \frac{7.9 \times 86^2 \times 2}{(118 - 44)^2}
\]

\[
n = \frac{116856}{5329}
\]

\[
n = 22 \text{ per group}
\]

Where:
- \(f = \) significance level multiplied by power
- \(\alpha = \) significance level
- \(\beta = \) power
- \(\sigma = \) standard deviation of mean of low FODMAP diet group in previous trial
- \(\mu_1 = \) mean of the low FODMAP diet group in previous trial
- \(\mu_2 = \) mean of the sham diet group in previous trial
Assuming a power of 80% and overall 2-sided significance of 5%, 44 participants would be required across both groups to detect a difference between the diets. Based on previous work by our group (Staudacher et al., 2012, Staudacher et al., 2017b), 15% attrition was assumed, leading to an overall sample size of 52 participants across both groups (26 per group).

4.2.1.6 Recruitment

All patients were recruited by the thesis author (SC) from Guy’s and St Thomas’ NHS Foundation Trust (n=23) and Barts Health NHS Trust (n=29) and were mainly identified via two routes. Firstly, doctors, specialist nurses and pharmacists identified potentially suitable and interested patients and referred them to the investigator in weekly gastroenterology outpatient and infusion clinics at the included hospitals. Furthermore, potentially suitable patients were identified in virtual clinics and multi-disciplinary meetings.

Secondly, gastroenterology specialist dietitians at Guy’s and St Thomas’ NHS Trust vetted dietetic referrals for patients with inactive IBD requiring advice for GI symptoms and these were regularly viewed by the investigator. Patients who appeared to meet the main inclusion criteria upon preliminary medical note screening were contacted by the investigator.

4.2.1.7 Randomisation

This was a randomised trial. Randomisation reduces selection bias and ensures balance of characteristics, which may influence study outcomes, across the study groups. Allocation bias can occur in treatment trials through allocating individuals with particular known and unknown characteristics to particular groups, via selectively enrolling participants into a trial based upon the next treatment allocation (Kahan et al., 2015). This can lead to biased estimates of the effect size of interventions.

Randomisation was stratified based upon diagnosis (CD or UC) and faecal calprotectin at screening (≤100 μg/g and >100 μg/g), resulting in four strata (CD, ≤100 μg/g; CD, >100 μg/g; UC, ≤100 μg/g; UC, >100 μg/g). Stratified randomisation ensures that certain participant characteristics, that may have an influence on treatment effect, are balanced across study groups (Doig and Simpson, 2005). Patients with CD and UC may respond differently to a dietary intervention given their distinct pathophysiologies, as illustrated by a systematic review demonstrating different effects of fibre supplementation in patients with CD and UC (Wedlake et al., 2014). Additionally, IBD activity could affect response to dietary interventions, as demonstrated in which children with CD entering remission following enteral nutrition had a lower clinical disease activity score at baseline than those who did not (Sigall-Boneh et al., 2014).
Randomisation occurred in blocks of eight participants. Block randomisation is commonly used to ensure equal numbers of participants are allocated to each group within a trial. In contrast to simple randomisation, this method reduces the chance that the groups contain unbalanced numbers of participants should the trial cease earlier than expected. However, a drawback of block randomisation in single-blinded trials is that it is easier for investigators to correctly guess the next group allocation, therefore compromising allocation concealment (Doig and Simpson, 2005). Larger block sizes mitigate this issue to some extent.

A random allocation sequence was prepared by a researcher not involved in trial recruitment (Dr Megan Rossi, Research Associate, King’s College London) using an online program (www.sealedenvelope.com), with a 1:1 ratio of low FODMAP to sham diet allocation. Group allocation for each trial ID was sealed in an opaque envelope, with separate envelopes for each stratum. Envelopes were stored in a locked filing cabinet at King’s College London and the next envelope was opened only after baseline data had been collected.

4.2.1.8 Blinding

Maximum blinding of participants and investigators is advisable in any treatment trial, particularly in trials of FGS which often involve behavioural interventions and use subjective end-points (Irvine et al., 2016). Blinding participants and investigators reduces conscious and unconscious bias associated with knowledge of treatment allocation. For example, ineffective blinding can alter expectation bias in participants in different treatment groups of a trial (Staudacher et al., 2017a). Blinding of dietary interventions is notoriously more challenging than that of pharmacological treatments.

Placebo ‘sham’ dietary advice was used in the control group in this trial. During screening, participants were informed that the purpose of the trial was to investigate a type of dietary intervention that may improve GI symptoms. For ethical reasons, patients were informed that they would be randomly allocated to either the ‘intervention’ diet group (described as the dietary advice under investigation) or the ‘placebo’ diet group (described as the control or comparison dietary advice). The appearance of the written resources for both the diet groups were similar, with the exception of the foods listed as ‘suitable’ and ‘unsuitable’. The terms ‘fermentable carbohydrates’, ‘the low FODMAP diet’ or mechanisms of the intervention were not mentioned in the study information, written resources or verbally by the investigator. In accordance with the use of the low FODMAP diet in clinical practice, both booklets provided a list of low and high fibre foods to ensure that participants maintained their habitual fibre intake. The dietary advice was provided in the same style for both groups and the same amount of time was taken to deliver it. To ensure similar efficacy of the diet to that seen in clinical practice, the
dietary advice was provided by the same registered dietitian trained in the low FODMAP diet (SC), who could therefore not be blinded to the allocation.

4.2.1.9 Compliance

Compliance to an intervention during a treatment trial can have a major impact upon its measured effectiveness (Yao et al., 2013) and may be influenced by prior patient beliefs and behaviours, socioeconomic issues, knowledge or suspicion of the treatment allocation, and side effects of the treatment. Trials involving whole diet interventions, such as the low FODMAP diet, require adherence to many dietary modifications in free-living individuals, which could limit compliance compared to pharmacological or nutrient trials that simply require taking a pill/supplement. Previous studies have demonstrated variable compliance to dietary interventions for functional bowel disorders, which likely reflects variable patient characteristics and measures of compliance (Osicka et al., 2015).

Measuring compliance to dietary interventions is usually via subjective participant ratings, which can be influenced by the participant’s perception of the desired response and by their perception of compliance, which may differ from that of the investigator. Nutritional biomarkers have been used as measures of compliance in, for example, fatty acid supplementation (Patterson et al., 2015) and whole diet interventions (Le Marchand et al., 1994), and reduce bias associated with subjective measures. However, to date there are no biomarkers for compliance to the low FODMAP diet. Therefore, in previous trials of the low FODMAP diet, compliance with the dietary intervention has been measured using food records and participant-reported ratings of compliance (Staudacher et al., 2012).

In this trial, overall participant-reported compliance was assessed with a question at the end of trial visit relating to compliance throughout the whole 4-week diet. Participants were asked to rate overall compliance to the trial diet in the previous week as: “never/rarely" (0-25% of the time), “Sometimes” (25-50% of the time), frequently (50-75% of the time) or “always" (75-100% of the time). For defining the patients to be included in the per protocol analysis, compliance with the diet was defined as reporting to have followed the diet ≥ 75% of the time throughout the trial.

To monitor and encourage optimal compliance, participants were also asked about compliance on a weekly basis during telephone contact with the investigator using the aforementioned responses. Additionally, participants were asked to recall dietary intake in the preceding 24 hours and this was also used to determine the correct compliance rating. Weekly compliance ratings were not used in the end of trial overall compliance assessment.
4.2.1.10 Adverse events and withdrawals

Adverse events were monitored by the investigator during weekly telephone contact with the participant throughout the 4-week trial, and at the end of trial visit. For each reported adverse event, onset, duration, frequency, intensity, management and possible relationship with the trial diet was recorded. Where the relationship with the trial diet was unclear, the principle investigator for the appropriate trial site was contacted for clarification.

Participants were free to withdraw at any time during the trial, and could do so without providing a reason (classified as having ‘withdrawn consent’). Where participants were not contactable for several consecutive weeks despite multiple attempts from the investigator, they were deemed ‘lost to follow up’ and were withdrawn from the trial. Additionally, participants meeting an exclusion criterion during the trial (for example commencing antibiotics, changing IBD medications or becoming pregnant) were withdrawn.

4.2.1.11 Ethics approval

Ethical approval for the trial was granted by the London – Dulwich Research Ethics Committee on 22nd October 2015 (15/LO/1684) and the RCT was registered on the ISRCTN registry on 21st January 2016 (ISRCTN17061468). No patients were recruited until ethical approval and trial registration were in place.

4.3 Trial protocol and procedures

The trial protocol and procedures are illustrated in Figure 4.1.

4.3.1.1 Screening 1

Patients expressing interest in the trial were screened against inclusion and exclusion criteria and, if eligible, were provided with a participant information sheet. They were invited to arrange a screening visit once they had read and understood the participant information sheet.

At the screening visit, informed consent was taken and patients were provided with a 7-day food and symptom diary and a whole stool collection kit for the subsequent baseline visit. The patient provided a stool sample for faecal calprotectin screening (analysed by the author of this thesis (SC), using the technique described in section 3.4.11.3) and a blood sample for CRP screening (analysed in the hospital pathology department). The food and symptom diary was completed for 7 days preceding the baseline visit.
4.3.1.2  Screening 2

On the final day of the 7-day food and symptom diary, patients were contacted to determine whether symptoms reported during the screening week met the threshold required for inclusion in the trial (not reporting adequate relief of GI symptoms, as well as abdominal pain, bloating or diarrhoea on at least two of seven days) (section 4.2.1.3). Patients reaching the symptom threshold were invited to return for a trial baseline visit the following day.

4.3.1.3  Baseline visit

Food and symptom diaries were checked for completeness and accuracy and data regarding smoking history, current medications and previous flares of IBD were collected and participants completed five questionnaires measuring various clinical outcomes (section 4.4). Body weight and height were measured.

Participants provided a whole stool sample as well as blood samples for immunology analysis and serum storage.

Following measurement of all baseline outcomes, the randomisation envelope was opened and the trial diet allocated to the patient was explained in detail by the thesis author (SC) with reference to the patient’s habitual diet as outlined in the 7-day screening diary.

4.3.1.4  Weekly monitoring

Participants were contacted weekly during the 4-week trial to address concerns and to monitor compliance and any changes in medication and smoking status.

4.3.1.5  End of trial visit

Participants provided a further 7-day food and symptom diary which had been completed during the fourth and final week of the trial diet. This diary was checked for completeness by the thesis author (SC) and dietary intake was reviewed. Any changes in medications or smoking history were documented and participants were asked whether they were aware of their diet allocation (intervention or placebo). Participants completed six questionnaires to measure various clinical outcomes and diet acceptability (section 4.9) and body weight was measured.

Participants provided a whole stool sample and blood samples were collected as in the baseline visit, with an extra vacutainer of blood collected for CRP measurement.

Following measurement of all outcomes, diet allocation was revealed to the participant and participants allocated to the low FODMAP diet group during the trial were provided with FODMAP reintroduction advice, in line with standard clinical practice. Participants allocated to
the sham diet group were provided with the low FODMAP dietary advice if they wished and a follow-up appointment was arranged with the investigator to discuss FODMAP reintroduction.

4.4 Outcome measures and rationale: clinical efficacy

4.4.1 Gastrointestinal symptoms and stool output

Gastrointestinal symptoms in patients with IBD and functional bowel disorders are extremely heterogeneous within and between individuals, and thus the accurate and meaningful measurement of these symptoms is challenging (Klein, 1988).

Irritable bowel syndrome is a disorder diagnosed and monitored through GI symptoms alone, and therefore a symptom measure used in clinical practice and research must accurately reflect patient’s symptoms and have the ability to detect clinically meaningful symptom responses (Irvine et al., 2006). Symptom measures in IBS are broadly classified as ‘global’ measures (e.g. Adequate Relief), individual symptom measures or composite symptom measures (Bijkerk et al., 2003). Ideally, symptom measures in IBS should span relevant elements of the operational definition (Klein, 1988), which according to the Rome IV diagnostic criteria are abdominal pain and an altered bowel habit (Mearin et al., 2016).

The concept of an overlap between FGS and IBD is relatively new, with the traditional view that the conditions are pathophysiologically and clinically distinct (Barbara et al., 2014). While there are clear similarities in GI symptoms experienced by patients with IBS and IBD (Quigley, 2016), there are likely fundamental differences in illness experiences and symptom perception in patients with IBD. The use of tools validated in patients with ‘true’ IBS in patients with IBD may therefore be problematic. However, to date there are no GI symptom measures validated specifically for use in patients with FGS in IBD. Although it is debatable whether patients with IBD can meet the Rome criteria for a functional bowel disorder (given the presence of an organic abnormality) (Quigley, 2016), in the absence of specific tools for this patient group IBS validated tools continue to be utilised (Halpin and Ford, 2012, Prince et al., 2016).

4.4.1.1 Rationale for choice of method: IBS Severity Scoring System (IBS-SSS)

The IBS Severity Scoring System (IBS-SSS) was used as a composite scoring system in the current study (Francis et al., 1997). The IBS-SSS is composed of four visual analogue scales (VAS), assessing the frequency and severity of abdominal pain, severity of bloating, satisfaction with bowel habits and interference of symptoms with life in general. Each question is scored out of 100, with a maximum total score of 500. Scores of 75-175 are deemed ‘mild’ IBS, 175-300 are ‘moderate’ and >300 are ‘severe’ (Francis et al., 1997). This scoring system has been validated across a combination of secondary and tertiary care patients and demonstrated reproducibility.
and sensitivity to a clinically meaningful change in symptoms following hypnotherapy (Francis et al., 1997). In a study comparing different functional symptom measures, the IBS-SSS performed well in parameters such as responsiveness, face validity, content validity and construct validity, although the practical utility was questioned given the difficulty in using VAS experienced by some patients (Bijkerk et al., 2003).

The IBS-SSS has several merits which made it appealing for use in this trial. It includes an assessment of both abdominal pain and bowel habits, the diagnostic criteria for IBS, and therefore the measure demonstrates content validity. Furthermore, it specifically measures abdominal pain rather than discomfort, in contrast to other IBS symptom questionnaires (Svedlund et al., 1988). The IBS-SSS includes an assessment of both abdominal pain severity and incidence. Severity of irritable bowel syndrome is considered by the Rome Foundation an important factor in determining patients’ illness experience and behaviour, and in guiding diagnostic decisions and treatment planning (Drossman et al., 2011). The IBS-SSS also includes a measure of the impact of symptoms on life in general, an important consideration in IBS treatment given the significant impact of IBS symptoms on quality of life (Chang, 2004). Finally, given that this measure provides an absolute score, a minimally clinically important difference (MCID) can be detected following treatment. A fall in absolute score of ≥50 points was determined to perform with optimal sensitivity and specificity to detect this MCID (Francis et al., 1997).

The attention given to abdominal pain in the IBS-SSS could be seen as both a strength and a limitation of this measure. Although pain is a cardinal symptom of IBS, other common symptoms such as flatulence and belching are omitted from the IBS-SSS. These symptoms can be equally or more debilitating for some patients than abdominal pain (Spiegel et al., 2010, Lembo et al., 1999).

### 4.4.1.2 Rationale for choice of method: Adequate relief

The global symptom measure of adequate relief was used in this trial. Global measures of GI symptoms are attractive for use in functional bowel disorders given the heterogeneous symptom profile experienced by patients and because they are simple to administer and interpret (Naliboff et al., 1999). Adequate relief, measured using the Global Symptom Question (GSQ), has traditionally been considered acceptable as the primary endpoint in functional bowel disorder trials (Irvine et al., 2006), and hence many pharmaceutical IBS trials have used this measure as the primary endpoint (AKEHURST and KALTENTHALER, 2001). A Rome Foundation working party showed equivalent construct validity of the GSQ when compared to a 50% improvement in pain, and both outcome measures were able to detect MCID (Spiegel et al., 2010, Lembo et al., 1999).
2009). Adequate relief has demonstrated efficacy of various drugs for IBS such as alosetron and cilansetron, where this measure performed equivalently to symptom-specific measures (Camilleri et al., 2007).

However, this type of outcome measure has faced criticism in recent years. Baseline symptom severity may confound a clinical response determined using this measure (Whitehead et al., 2006), and it is not sensitive to changes in individual symptoms or different symptom responses in different IBS subtypes. Furthermore, since it is a dichotomous outcome, it may not always be capable of detecting a deterioration in symptoms (Camilleri et al., 2007). These concerns have led the US Food and Drug Administration (FDA) to discourage the use of global symptom measures as primary end-points in clinical trials of FBD (Irvine et al., 2016).

Nonetheless, based on the merits of this measure described above, many IBS trials continue to use it and it arguably reflects an improvement in overall symptoms important to the individual patient (Camilleri et al., 2007), therefore it was used in this trial.

### 4.4.1.3 Rationale for choice of method: Gastrointestinal Symptom Rating Scale (GSRS)

The gastrointestinal symptom rating scale (GSRS) was used to measure individual symptoms in the current trial. While the IBS-SSS provides a measure of some GI symptoms, several potentially relevant symptoms are omitted, such as faecal urgency, flatulence, fatigue and specific changes in stool output (Spiegel et al., 2010). The GI symptom rating scale (GSRS) is a modified Likert scale (Svedlund et al., 1988, Revicki et al., 1998, Wiklund et al., 2003). A study comparing the psychometric and methodological characteristics of IBS symptom measures concluded that the GSRS demonstrates internal consistency, face validity, content validity and construct validity (Bijkerk et al., 2003). In a trial of 234 patients with IBS, the GSRS demonstrated test-retest reliability, was able to discriminate different symptom clusters and was responsive to changes in symptoms (Wiklund et al., 2003).

The GSRS includes categorical responses for symptom severity, with the variation used in this trial including 'absent', 'mild', 'moderate' and 'severe'. This carries the inherent limitation that, in contrast to VAS, patients are forced to choose from limited responses, when in fact their answer may lie between the possible responses. This may therefore compromise sensitivity and poses potential challenges in statistical analysis in converting categorical responses to numerical scores (Naliboff et al., 1999).

### 4.4.1.4 Rationale for choice of method: Bristol Stool Form Scale

Stool frequency and consistency was measured using the Bristol Stool Form Scale. Stool consistency is an important component of stool output and refers to viscosity and rheology of
stool. Stool consistency is mainly determined by water content, with the ratio of faecal water to solids remaining within a narrow range in healthy individuals despite variation in stool volume (Wenzl et al., 1995). Furthermore, stool consistency correlates with the proportion of stool water in patients with chronic diarrhoea (Wenzl et al., 1995). An evaluation of stool output in healthy individuals showed that 33% of women and 40% of men regularly pass stool once a day (Heaton et al., 1992). Dietary habits can influence stool frequency and consistency. For example, more frequent and softer stools are passed by healthy individuals following a vegan diet compared to those following an omnivorous diet (Davies et al., 1986). It was especially important in this trial, given that altered stool output is required for a diagnosis of IBS (Mearin et al., 2016) and is a common feature of IBD (Mowat et al., 2011).

Stool water content, viscosity and rheology can be measured directly using lyophilisation, a viscometer and a penetrometer, respectively (Bliss et al., 1999, Wenzl et al., 1995, Davies et al., 1986). However, this poses clear logistical challenges in treatment trials, in which it may be desirable to assess stool consistency prospectively over several days or weeks. In these circumstances, stool shape and texture may instead be assessed visually and recorded by participants (Bliss et al., 1999). Stool form scales have been developed for this purpose, and the most commonly used in the UK is the Bristol Stool Form Scale (BSFS) (O'Donnell et al., 1990). This scale has been utilised for a number of years in research and clinical practice, and has been adapted for use in children (Lane et al., 2011) and translated into a number of languages (Chira and Dumitrascu, 2015, Pares et al., 2009, Martinez and de Azevedo, 2012). However, despite its widespread use and recommendation for use by the Rome Foundation (Mearin et al., 2016), the BSFS has only recently undergone extensive validation (Blake et al., 2016).

Discrepancy between recalled and recorded stool frequency and consistency has been observed in patients with IBS (Coletta et al., 2010). Furthermore, poor concordance in stool output was observed when measured with a 4-week diary compared to a retrospective questionnaire in healthy adults (Bellini et al.). Therefore in this trial, the form of each stool passed during the week prior to the baseline visit and the week prior to the end of trial visit was recorded prospectively (and therefore stool frequency was also measured) to minimise recall bias.

### 4.4.2 Clinical disease activity

The goal of therapy in IBD is to manage inflammation effectively in order to improve quality of life and reduce the risk of long-term complications, while minimising the risks associated with therapy. There are three main factors that must be considered in the assessment of IBD activity: the overall impact of current symptoms on the patient, the extent and severity of inflammation and any damage that inflammation has inflicted on the GI tract (Peyrin-Biroulet et al.).
Assessment of IBD activity can include clinical, biochemical, endoscopic, radiologic and histologic measures (Walsh et al., 2016).

Clinical activity indices are based exclusively or partially on GI and extra-intestinal symptoms. The first clinical disease activity index for Crohn’s Disease was developed in 1976 to enable consistent assessment of disease activity in Crohn’s disease clinical trials (Best et al., 1976) and several alternatives have subsequently been developed, in addition to UC disease activity indices (Walsh et al., 2016).

4.4.2.1 Rationale for choice of method: Harvey-Bradshaw Index (HBI)

The Harvey Bradshaw Index (HBI) was used to assess clinical activity of CD in this trial. The HBI is a short disease activity index for CD and comprises five questions, assessing general well-being, abdominal pain, liquid stools, abdominal mass and extra-intestinal symptoms in the preceding 24 hours (Harvey and Bradshaw, 1980). This is simple to administer and is suited to clinical trials. The Crohn’s Disease Activity Index (CDAI) is based upon the number of liquid stools, abdominal pain and overall well-being measured across 7 days. The CDAI continues to be the most widely used instrument for the assessment of CD activity in clinical trials, however it is rarely used in clinical practice due to the logistical challenges and complexity associated with completing and scoring the instrument (Walsh et al., 2016). Furthermore, significant inter-observer variability has been noted in the use of the CDAI (Sands and Ooi, 2005). Although the HBI omits several components included in the CDAI, results of the two instruments display a strong positive correlation (Harvey and Bradshaw, 1980, Vermeire et al., 2010). Furthermore, results of the HBI appear to display less variability than the CDAI (Jorgensen et al., 2005).

Although a 7-day GI symptom diary was completed prior to enrolment in the current trial, it was felt that certain components of the CDAI would be difficult for the investigator to obtain (e.g. recent haematocrit may not be available since patients were in remission). Therefore, given the aforementioned strong correlation between the CDAI and the HBI, the HBI was used for assessing CD activity at the baseline and end of trial visits.

4.4.2.2 Rationale for choice of method: Partial Mayo Score

The Partial Mayo Score was chosen to measure UC disease activity in the current trial since it is simple to administer and is derived from the Mayo Score, the most widely used invasive disease activity index (Su et al.). This is in contrast to the case-control study described in Chapter 2 of this thesis (section 2.4.3), where a Physician Global Assessment (PGA) was not always possible due to the recruitment methods (for example, from virtual gastroenterology clinics). Thus, in the
case-control study the Partial Mayo score was deemed unfeasible and the SCCAI was used to assess UC activity.

There are 13 clinical disease activity indices for UC, eight of which are based purely upon GI and extra-intestinal symptoms (Walsh et al., 2016). The Partial Mayo Score, comprising the non-endoscopic components of the Mayo Score (Lewis et al., 2008), is the most commonly used clinical disease activity index in clinical trials of UC. This instrument includes an assessment of stool frequency and rectal bleeding and a Physician Global Assessment of disease activity. Although the Partial Mayo Score has not been extensively validated, it has been shown to optimally differentiate patients with active and inactive UC compared to other UC disease activity indices and displayed construct validity and responsiveness to change. However, the instrument did not shown sufficient test-retest reliability (Turner et al.).

4.4.3 Patient-reported outcome measures

Patient-reported outcomes measures (PROMs) assess patients’ experiences with a disease and are increasingly used to assess the effectiveness of interventions in research and clinical settings (El-Matary, 2014). The use of PROMs is advocated by the Medical Research Council, although a workshop in 2009 identified on-going research gaps surrounding them, including the content of PROMs, gaps in the current repertoire of PROMs and the need to develop guidelines for the use of PROMs (MRC, 2009). Debate around the value of PROMs in determining disease improvement continues and some healthcare professionals remain opposed to them as the predominant indicator of improvement, due to a lack of objectivity and a host of confounding factors that may influence scores (El-Matary, 2014). In contrast, many healthcare professionals recognise the potential advantages of the routine use of PROMs, such as the positive effects that may be gained by involving a patient in their own care, and the elimination of observer bias (Black and Jenkinson, 2009).

4.4.3.1 Rationale for choice of method: IBD control questionnaire

The IBD control questionnaire was used as PROM in this trial (Bodger et al., 2013). Development of this instrument included a literature search of existing PROMs and a qualitative study consisting of patient focus groups. The resulting instrument was validated in patients with IBD and demonstrated good internal consistency, test-retest reliability, construct validity, ability to discriminate between mild, moderate and severe IBD, and responsiveness to change in IBD activity (Bodger et al., 2013). Patients with IBD had lower scores in each of the PROMIS domains than the general population.

This trial aimed to assess the effect of the low FODMAP diet in inactive IBD and was therefore not targeted at improving disease activity. Nonetheless, it was of interest to measure patient-
perceived IBD control before and after the intervention, with the hypothesis that an improvement in GI symptoms may lead to a greater patient-perceived control of IBD. Since symptoms of active IBD and IBS-like symptoms overlap, it may be difficult for some patients to distinguish between them.

### 4.4.4 Health-related quality of life

Both IBD and IBS substantially impact upon health-related quality of life (HR-QOL) (Akehurst et al., 2002, Rubin et al., 2004). Some studies have demonstrated differences in HR-QOL between IBD and IBS (Blagden et al., 2015, Tkalcic et al., 2010), while others have shown similar HR-QOL between the conditions (Pace et al., 2003). The impact of IBD on HR-QOL may be influenced by disease activity (Graff et al., 2006), gender, psychological distress, frequency of relapses and frequency of hospitalisations (Moradkhani et al., 2013).

It is unsurprising that HR-QOL is consistently shown to be poorer in patients with IBS-like symptoms in inactive IBD compared to patients without IBS-like symptoms (Simrén et al., 2002, Jonefjall et al., 2013, Abdalla et al., 2017, Farrokhyar et al., 2006, Ansari et al., 2008), and may be impaired to a similar degree as in active IBD (Jonefjäll et al., 2016, Ansari et al., 2008). The impairment in HR-QOL and increased anxiety and depression associated with IBS-like symptoms in inactive IBD have been shown to associate with the number of GI symptoms (Bryant et al., 2011). Impaired HR-QOL may also be associated with disease type (CD vs. UC), annual income and smoking status (Farrokhyar et al., 2006). The cross-sectional nature of the majority of studies to date precludes any firm conclusions regarding the direction of association between IBS-like symptoms and HR-QOL in IBD. However, an improvement in certain domains of HR-QOL has been demonstrated in patients with IBS following low FODMAP dietary advice (Staudacher et al., 2017b), therefore this diet may improve HR-QOL in inactive IBD and is an important outcome for assessment.

#### 4.4.4.1 Rationale for choice of method: UK IBD Questionnaire (UK IBDQ)

The UK inflammatory bowel disease questionnaire (IBDQ) was used to assess HR-QOL in this trial (Cheung et al., 2000). Some HR-QOL assessment tools are generic measures, purported to be suitable across a range of diseases, populations and treatments (Patrick and Deyo, 1989). Generic instruments allow a comparison of HR-QOL across different disease states; for example, the Sickness Impact Profile produces different scores for a range of chronic diseases including IBD (Patrick and Deyo, 1989). However, they lack content validity when used in specific diseases, since there are few or no items related specifically to the major domains of HR-QOL that may be specifically impaired in different diseases (Patrick and Deyo, 1989). Instruments have therefore been developed to include factors relevant and important to patients with specific diseases,
including chronic obstructive pulmonary disease and rheumatoid arthritis (Weldam et al., 2013, Lee et al., 2014b). Generic and disease-specific HR-QOL instruments likely measure different aspects of HR-QOL, as demonstrated by a study showing a lack of correlation in HR-QOL scores on generic and disease-specific measures completed contemporaneously by patients with lung disease (Assari et al., 2009).

At least ten IBD-specific HR-QOL instruments currently exist (Alrubaiy et al., 2015). The McMaster IBDQ is the most widely used instrument and meets all of the criteria for desirable measurement properties, some of which are lacking in other instruments (Alrubaiy et al., 2015). The IBDQ assesses bowel, social and emotional function and systemic symptoms and demonstrates validity, internal consistency, reliability and responsiveness to change (Guyatt et al., 1989, Irvine, 1999). The IBDQ has been translated into several different languages (Ren et al., 2007, Janke et al., 2005) and has been adapted and validated for use in a UK population (Cheung et al., 2000). This adaptation was initiated by investigators planning a UK RCT, with a consensus that the wording of the McMaster IBDQ was ambiguous in places and the responses too complex.

4.5 Outcome measures and rationale: microbiota and markers of fermentation

4.5.1 Sample collection

An important outcome in this RCT was the impact of the low FODMAP diet on the GI microbiota. There are differences in microbiota composition between the luminal and mucosal microbiota in healthy controls and patients with IBD (Ringel et al., 2015, Chen et al., 2014). Although the majority of studies have examined the faecal microbiota in response to treatment due to obvious logistical challenges associated with obtaining biopsies, the mucosa-associated microbiota are in closer proximity to host epithelial and immune cells and could therefore have a greater interaction with the host. However, the bowel preparation required for endoscopic procedures such as colonoscopy and flexible sigmoidoscopy is known to impact significantly upon the mucosal microbiota in healthy controls and patients with IBD (Shobar et al., 2016). The luminal microbiota may also be more susceptible to alterations in dietary intake than the mucosal microbiota, which receives a more consistent nutrient balance (Donaldson et al., 2016). Faecal samples were collected at baseline and end of trial to examine the luminal microbiota.

The descending and sigmoid colon are predominantly anaerobic and a gradient of microbes associated with oxygen distribution has been observed in mice and humans (Albenberg et al., 2014). Therefore, exposure of faecal samples to the external environment after voiding has the potential to alter the microbiota composition and/or function. *In vivo* sampling would overcome this issue but is extremely logistically challenging. Furthermore, a strong correlation has been
observed in diversity and abundance of the luminal microbiota between faeces obtained directly from the sigmoid colon lumen and that passed by patients (Couch et al., 2013), although stool samples were stored with a substance to maintain an anaerobic atmosphere after voiding and prior to freezing. The impact of exposure to the external environment and the length of time required for changes to occur in other luminal parameters such as SCFA and pH are unclear.

Stool sample collection was carried out by all participants as per the International Human Microbiome Standards standard operating procedure 2 (V2) (IHMS, 2015a), in order to comply with standards for studies performing metagenomic sequencing. Stool samples were collected by patients at the baseline and end of trial visits using the stool collection kit provided and explained by the investigator (Appendix 8.19). A thick stomacher bag was used to line an opaque plastic container, into which a whole stool was passed. The bag was then folded on top of the sample and the container was sealed with a secure plastic lid. Participants were asked to avoid urinating while passing the sample. Participants were permitted to collect the sample within two hours prior to the baseline visit, at which point it was placed on ice until processing. Temporary refrigeration of faecal samples has been shown to maintain microbiota composition similarly to freezing at -80°C (Choo et al., 2015), therefore if the sample was being transported from home or work to the research site, the participant was asked to place the sample on ice during transport. Alternatively, the sample was collected during the visit and placed on ice until processing.

Following the trial visit, the stool sample was processed by the investigator. Bacteria in stool are structured and spatially organised and random samples taken from different areas of un-homogenised stool samples leads to intra-individual variation in microbiota (Hsieh et al., 2016, Gorzelak et al., 2015). This intra-individual variation is significantly reduced by homogenisation prior to analysis, therefore stool samples were homogenised for 2 minutes on each side prior to further processing (Steward Laboratory Blender Stomacher 400).

Immediately after processing, faecal aliquots were frozen at -80°C, since this has been shown to preserve taxonomic composition compared to DNA extraction on fresh stool (Fouhy et al., 2015) and is therefore recommended for faecal sample storage (Thomas et al., 2015).

### 4.5.2 Metagenomic sequencing

Stool sample preparation prior to storage was performed by the author of this thesis (SC), while DNA extraction and metagenomic sequencing was performed at Institut National de la Recherche Agronomique (INRA) (Jouy-En-Josas, Paris, France), in the laboratory of and led by Professor Dusko Ehrlich. Regular contact with SC and Professor Kevin Whelan was maintained during laboratory analysis and SC visited INRA for one week to learn about and be trained in the
techniques used and to guide the bioinformatics analysis. The pipeline used for metagenomic sequencing and taxonomic assignment on these samples is illustrated in Figure 4.2. For the purpose of this thesis, functional annotation of the microbiome is not included.

4.5.2.1 Rationale for choice of method

The characterisation of the compositionally and functionally complex GI microbiota began with culture-dependent techniques that are limited to cultivable bacterial species, dependent upon pH, redox state, temperature and nutrient availability of the growth media, and estimated to represent <20% of the environmental microbial consortia (Ward et al., 1990). These studies therefore had the inherent limitation that the majority of the GI microbial community remained uncharacterised. Initial cultivation studies of GI bacteria failed to detect obligate anaerobes, which have since been determined to constitute a large proportion of the GI microbiota. Although anaerobic culture techniques have since been developed, the complexity and varied growth requirements of the GI microbiota is such that culture-dependent techniques continue to limit the proportion of species that can be characterised (Rajilić-Stojanović and de Vos, 2014).

The development of molecular techniques such as quantitative polymerase chain reaction (qPCR) and fluorescence in situ hybridisation (FISH) have progressed the field of GI microbiota characterisation. The majority of these methods utilise the small subunit (16S) ribosomal RNA, which has highly conserved regions and regions that vary with evolutionary time (Tringe and Hugenholtz, 2008). This can therefore be used as a marker of all bacteria and can identify individual bacterial species based upon the hypervariable regions of the 16S rRNA gene. These techniques are however limited to species for which hypervariable 16S rRNA sequences are known for the development of primers (qPCR) or probes (FISH). PCR bias is a further limitation of qPCR. Furthermore, even if the 16S sequences were known for all GI bacteria, qPCR and FISH would be prohibitively time and labour intensive for an exhaustive assessment of the GI microbiota.

The development of next-generation sequencing techniques has proven revolutionary for the characterisation of the GI microbiota. 16S rRNA gene sequencing and mapping to databases of known sequences permits comprehensive taxonomic assessment of the GI microbiota. However, results may vary depending upon the 16S region sequenced (Rintala et al., 2017) and PCR bias remains a problem. Furthermore, an individual bacterium can possess copies of the 16S rRNA gene with different sequences which can thus over-estimate bacterial diversity. Additionally, sequencing only one or two hypervariable 16S rRNA regions makes it challenging to distinguish species, and different regions sequenced in different studies introduces challenges
in comparing studies (Thomas et al., 2015). Finally, 16S rRNA sequencing does not allow identification of strains within a species that may vary considerably in terms of function.

Shotgun metagenomic sequencing is a powerful method of characterising microbiome composition, in which all DNA is sheared into fragments and then sequenced. In contrast to 16S sequencing, this technique does not require extensive DNA amplification and thus minimises PCR bias. It does not rely on a single target gene to assign taxonomy and therefore more accurately captures microbiome composition than 16S sequencing (Thomas et al., 2015). Furthermore, the full genetic potential of the GI microbiome can be characterised, allowing an assessment of not only composition but also potential functions of the GI microbiome. For example, genes involved in carbohydrate metabolism may already be altered in IBD (Erickson et al., 2012) and could feasibly be impacted by dietary carbohydrate manipulation. Metagenomic sequencing does not, however, measure gene expression. Additionally, the DNA extraction protocol has a significant influence on metagenomic analyses, greater than variations in sample storage and library preparation (Costea et al., 2017). The cost of metagenomic sequencing is greater per sample than the aforementioned methods, although this is declining due to greater efficiency of newer sequencing platforms. Metagenomic sequencing generates vast quantities of complex data that requires specialist bioinformatics pipelines to produce meaningful conclusions, therefore in this trial metagenomic sequencing was performed by experts in the technique and bioinformatic analysis at INRA.

4.5.2.2 Sample preparation and storage

Stool sample preparation for metagenomic sequencing analysis was performed in line with the International Human Microbiome Standards (IHMS) standard operating procedures for metagenomic sequencing studies (IHMS, 2015b). These guidelines aim to maintain consistency in sample preparation, DNA extraction and sequencing, thus providing optimal comparability between metagenomics studies conducted in different research centres.

Within 2 hours of stool collection, 0.2-0.5 g aliquots of fresh, homogenised stool were transferred in triplicate to sterile screw-cap 2 ml micro-tubes (Sarstedt AG and Co, Nümbrecht, Germany). The aliquots were stored at -80°C for 1-14 months, as this has been shown to preserve DNA of certain bacterial species (Fouhy et al., 2015). Samples were then shipped on dry ice to INRA on 31st May 2017 for DNA extraction and shotgun metagenomic sequencing.
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Figure 4.2 The metagenomic sequencing pipeline used in this trial. Fresh faecal samples were collected and stored at -80°C (1). Following thawing, DNA was extracted using the Sambo platform (2) and DNA fragment libraries were constructed (3). These were sequenced using the MetaQuant platform (4). After mapping to the reference gene catalogue, gene frequency tables were obtained (5) and taxonomic assignment is performed using 50 marker genes per species (6). Data analysis was then performed (7).

4.5.2.3 DNA extraction protocol

The Sambo platform was used for DNA extraction from the stool samples prior to metagenomic sequencing using a standardised protocol published by IHMS (SOP 07 V2). This was carried out in the laboratory of Professor Dusko Ehrlich at INRA (Jouy-En-Josas, Paris, France), as follows:
Day 1

1. 250 μL Guanidine Thiocyanate, 40 μL of 10% N-lauroyl sarcosine and 500 μL 5% of N-lauroyl sarcosine were added to each tube containing ~ 200 mg faeces
2. Tubes were vortexed until fully homogenised, followed by a 60 minute incubation at 70°C in a dry bath
3. Each tube was vortexed on high speed with 750 mg of 0.1 mm glass beads and shaken to mechanically disrupt the cells with a Bead Beater™ for 10 minutes total
4. Each tube was vortexed with 15 mg of polyvinylpolypyrrolidone (PVPP) powder and then centrifuged at 14,000 rpm (18,000 g) for 5 minutes at 4°C.
5. The supernatant was transferred to a sterile, graduated 2 ml tube and set aside
6. The remaining pellet was vortexed with 500 μL of TENP (containing Tris, EDTA, NaCl, PVPP) and centrifuged at 14,000 rpm (18,000 g) for 5 minutes at 4°C. The supernatant was added to that obtained in step 5 and two more washes with TENP were performed.
7. The supernatant was centrifuged at 14,000 rpm (18,000 g) for 5 minutes at 4°C and dispensed into two 2 ml Eppendorf tubes, producing two tubes per sample, to which 1 ml of isopropanol (propanol-2) was added and mixed gently.
8. Tubes were incubated for 15 minutes at room temperature, centrifuged at 14,000 rpm (18,000 g) for 5 minutes at 4°C and the supernatant removed from the tubes, retaining the pellet inside.
9. 450 μL of phosphate buffer and 50 μL of potassium acetate were added to one of the tubes, and the pellet and solution was added into the second tube and dissolved through gentle aspirations with a P200 pipette.
10. Samples were left on ice for at least 90 minutes, before centrifuging at 14,000 rpm (18,000 g) for 5 minutes at 4°C
11. The supernatant, containing DNA, was transferred to a 2 ml Eppendorf tube and 2 μL of RNase (10 mg/ml) was added and then vortexed briefly.
12. Tubes were incubated for 30 minutes at 37°C after which 50 μL sodium acetate and 1 ml 100% ethanol was added.
13. Tubes were mixed gently and stored overnight at -20°C.

Day 2

1. The samples were centrifuged at 14,000 rpm (18,000 g) for 5 minutes at 4°C and 1 ml 70% ethanol was added, followed by a further 5 minute centrifuge and removal of the ethanol.
2. The samples were centrifuged with 500 μL 70% ethanol at 14,000 rpm (18,000 g) for 5 minutes at 4°C and then the ethanol was removed.

3. The DNA pellet was dried for 10 minutes under a laminar flow hood and then dissolved in 200 μl of TE (containing Tris.HCl and EDTA).

Quality control was performed following DNA extraction and prior to library construction. Adequate quality of the DNA, defined as being 20 kilo base pairs, was determined using 1% agarose gel. At least 3 μg DNA was required for subsequent fragmentation and this was determined using NanoDrop.

4.5.2.4 Library construction and shotgun metagenomic sequencing

Metagenomic sequencing was performed via the MetaQuant platform in the laboratory of Professor Dusko Ehrlich at INRA (Jouy-En-Josas, Paris, France).

DNA of adequate quality and quantity was sheared into fragments of approximately 150 base pairs (BP) using the Covaris E220 system, which utilises Adaptive Focussed Acoustics ™ (AFA) technology. Agencourt beads (Beckman Coulter) were then used to purify fragments by removing residual nucleotides, salts and impurities.

A quality control step followed DNA fragmentation and consisted of determining fragment size, which was required to be approximately 150 bp for optimal sequencing with the Ion Proton technology. The size of DNA fragments was analysed using a size profile Fragment Analyser™ (Advanced Analytical), which is based upon parallel capillary electrophoresis and utilises fluorescent intercalating dyes to detect the quantity of different sized DNA fragments. DNA was quantified again using fluorescence emission using the Invitrogen Qubit™ Fluorometer (ThermoFisher Scientific). This is an efficient, highly sensitive and accurate assay suitable for use with very small or dilute samples. The assay utilises dyes that are specific for DNA and emit fluorescence only when bound to DNA. This is in contrast to measuring UV absorbance (the method utilised with the NanoDrop technology), which has lower sensitivity for discriminating between RNA and DNA. This method of DNA quantification is considered useful for next-generation sequencing since it quantifies intact double-stranded DNA.

A fragment library was then constructed for each sample. This was performed using the 5500 SOLID 48 Fragment Library Amplification Module and Ion Plus Fragment Library Adapters (Life Technologies). Following end-repair of the DNA fragments, adapters containing one of 96 single barcodes (Life Technologies) were ligated to each fragment. The constructed library was then amplified using PCR to ensure an adequate signal for the detection of all reads, followed by a further purification step and quantification using the 2100 Bioanalyzer System (Agilent). This
quantification step ensured that the library contained the target quantity of 90 pM DNA. The size of the DNA fragments was again assessed, aiming for 250 bp including adaptors.

For sequencing, purified and amplified DNA fragment libraries were loaded onto the Ion Proton sequencer (Life Technologies) and a minimum of 20 million high-quality reads were generated for each library. This technology utilises a chip containing 165 million wells, into which one bead is placed. To this bead, one DNA fragment should be ligated via the adaptors added during library construction. Free nucleotides are added sequentially to the wells (e.g. first adenosine, then thymine, etc). When DNA polymerase incorporates a nucleotide into a strand of DNA, a hydrogen ion is released. This hydrogen ion carries a charge that is detected by the Proton System, essentially a sensitive, small-scale pH meter. This allows each incorporated base to be detected, thus producing a DNA sequence. In contrast to Illumina sequencing technology, Ion Proton technology is quicker and permits the detection of errors at an earlier stage, thus reducing costly losses.

4.5.2.5 Bioinformatics and statistical analysis

Following sequencing of the microbial DNA fragment libraries, bioinformatics analysis was performed by Sébastien Fromentin (Biostatistics Analyst) and Dr Nicolas Maziers (Data Analyst) at INRA (Jouy-En-Josas, Paris, France).

First, sequencing reads were trimmed, cleaned and filtered for human DNA. Subsequently, the following bioinformatics steps were performed prior to data analysis:

1. Sequencing reads of varying length were mapped using the METEOR pipeline to the Integrated Gene Catalogue (IGC), which contains 9.9 million non-redundant genes including those derived from the microbiome of patients with IBD (Li et al., 2014b). Mapping was performed with a >95% identity threshold to account for gene variability. This mapping procedure resulted in a table of gene frequencies per sample.

2. Sequencing reads were normalised for varying lengths of genes using reads per kilobase of transcript per million mapped reads (RPKM). The number of reads uniquely mapping to a gene in the catalogue was divided by its nucleotide length, and the resulting normalised gene abundance was then divided by the total number of uniquely mapped reads for that sample. The resulting gene abundances were used as the microbiome profile for that individual.

3. Following mapping to the IGC, mapped read counts were plotted for each sample. Downsizing of the data was performed to adjust for varying sequencing depths among the samples. This was performed by setting an optimum threshold according to the
plotted curve, which equated to 14 million randomly-selected reads per sample. This read count was used for each sample in the subsequent analysis.

Upon completion of the above pre-analysis steps, metagenomic species (MGS) were identified within each sample. These are groups of >500 genes that have been shown to identify a microbial species due to co-varying abundance across samples (Nielsen et al., 2014). Relative abundance of each MGS was calculated as the mean abundance of the 50 most representative marker genes per species with correlating normalised abundance (Nielsen et al 2014). These known marker genes were mapped to the gene frequency tables produced during the catalogue mapping described above. To estimate the abundance of each gene within a species, it was assigned a relative abundance, with total gene abundance equating to a value of 1. Within a sample, the relative abundance of each species was calculated as the average abundance of the 50 marker genes for that species.

The gene richness of each sample was determined as the number of uniquely mapped reads, described above. Species alpha diversity was calculated using the Shannon Index, which accounts for both abundance and evenness of the species within a sample.

For statistical analysis, only participants with an available sample at baseline and end of trial were included. Gene richness, alpha and beta diversity and relative species abundance were compared between diet groups at end of trial using a likelihood ratio test (described in section 4.10). Paired analyses were also performed to assess the change in the above parameters between baseline and end of trial in each group. Sub-group analyses for each variable was performed for CD and UC. Exploratory analyses, including correlation analysis and principle component analysis (PCA), were performed to assess the gene and species richness and composition of responders and non-responders to the low FODMAP diet at baseline and end of trial, as well as according to faecal calprotectin concentrations.

4.5.3 Stool SCFA

Sample preparation was performed by the thesis author (SC) and GLC was conducted by the thesis author (SC) at King’s College London together with Robert Gray (Analytical Chemist) and supervised by Professor Kevin Whelan.

4.5.3.1 Rationale for choice of method

Short-chain fatty acids are a major product of bacterial fermentation in the human GI tract. Total SCFA concentrations rise rapidly in the caecum, where undigested carbohydrates are abundantly available for bacterial fermentation, and then progressively decline throughout the colon due to depletion of carbohydrates and rapid absorption by colonocytes (Cummings et al., 1987).
Several approaches have been used to directly measure in vivo SCFA concentration, including extraction of GI contents from sudden death victims (Cummings et al., 1987), measurement of SCFA concentrations in portal venous blood during surgery (Peters et al., 1992) and SCFA measurement in transverse or sigmoid colostomy effluent (Mitchell et al., 1985). In vivo SCFA production can also be determined by directly measuring the quantity of undigested carbohydrate entering the colon and the SCFA concentration in the colon. Furthermore, the difference between portal and venous blood SCFA concentration would indicate SCFA production (Millet et al., 2010). Access to the proximal colon is challenging and all of the aforementioned methods of in vivo SCFA measurement are invasive and technically challenging to perform, making them infeasible in the majority of RCTs.

The majority of SCFA are absorbed along the length of the colon, therefore faecal concentrations do not accurately represent SCFA production in the caecum. Furthermore, SCFA concentrations in faeces can be influenced by whole gut transit time (Lewis and Heaton, 1997a). Nonetheless, measurement of SCFA concentrations in faeces is less invasive than in vivo measurement and SCFA excretion can indicate changes in SCFA balance in the GI tract (production-absorption).

Gas liquid chromatography was used to quantify faecal SCFA in this trial. Other methods for quantifying SCFA include high performance liquid chromatography, nuclear magnetic resonance and capillary electrophoresis. Gas liquid chromatography is the most commonly used method for SCFA quantification and is based upon compound separation, relying on a carrier gas which transports the compounds within the sample towards a detector. Depending on properties such as size, different compounds have different retention times and thus elute from the oven column and reach the detector at different times, allowing assessment of the compounds within the sample (Primec et al., 2017). Gas liquid chromatography is quick, inexpensive and concentration of SCFA in the sample can be determined by calculating the area under the detection peak for each SCFA.

### 4.5.3.2 SCFA analysis protocol

Following homogenisation, duplicate aliquots of 3-5g of fresh stool were stored in sterile containers at -80°C for SCFA analysis. Extraction of SCFA was performed for all samples as follows:

1. Glass beads were placed into a 15 ml falcon tube together with 2-3 g of thawed faeces and SCFA extraction buffer was added in a 1 in 5 dilution (e.g. 2g of faeces to 8ml buffer).

SCFA extraction buffer (made up to 100 ml with distilled water):
0.1g mercury chloride (Sigma, UK) to inhibit bacterial fermentation within the sample (which would elevate SCFA concentrations)

1g phosphoric acid (Merck, Germany)

4.5 mg 2,2-dimethylbutyric acid (internal standard; Sigma, UK). The internal standard is not a product of the GI microbiota but exits the column within the same time as the SCFA’s of interest.

2. The faeces and buffer were vortexed for 2 minutes or until the slurry appeared fully homogenised, and the slurry was centrifuged at 5000 g (Beckman J2-HS, USA) at 4°C for 45 minutes.

3. Approximately 1 ml of the supernatant was passed through a sterile 0.2 µm filter into a Micro-vial Snap Ring Vial and stored at -20°C until gas liquid chromatography (GLC) analysis.

Prior to GLC analysis, extracted SCFA samples were defrosted at room temperature. A 9890A series GLC system (Agilent Technologies, US) was then used for SCFA analysis. This system is equipped with a flame ionisation detector and a 220 µm internal diameter, 25 m fused silica capillary column with a film thickness of 0.25 µm (ID-BP21, SGE, Australia). The system uses nitrogen as a carrier gas. The oven temperature began at 80°C, then increased 10°C per minute until 145°C, and 200°C at 100°C per minute. A total of 0.2 µl of sample was injected, with 1.2% formic acid cleaning solution (Merck, Germany) injected between samples to minimise carry over from the previous sample.

Gas liquid chromatographer calibration was performed with a blend of pure SCFA solutions at six different concentration, producing calibration curves (area vs concentration). The concentrations of acetate, propionate, butyrate, valerate, isobutyrate and isovalerate were read in duplicate for each sample. Mean concentration for each SCFA, for each patient, at each timepoint was then calculated (µmol/g wet weight). The individual SCFA concentrations were summed to give total SCFA concentration in each sample. Data analysis was performed using the Agilent Chromatogram database (Agilent Technologies, US).

4.5.4 Stool pH

Sample preparation and measurement of stool pH was performed by the investigator (SC) contemporaneously with stool processing.

4.5.4.1 Rationale for choice of method

The pH of the colonic lumen is determined by bacterial fermentation of undigested carbohydrates, proteins and amino acids, producing lactate, SCFA and alkaline compounds such as ammonia. Direct measurement of luminal GI pH using a radiotelemetry capsule in healthy
volunteers revealed a sharp drop in luminal pH from 7.5 in the ileum to 6.4 upon entering the caecum, due to a peak in bacterial fermentation in the caecum. This was then followed by a gradual rise in pH along the distal colon as SCFA are absorbed (Evans et al., 1988). The relevance of colonic pH to GI health remains unclear but potential roles in colon cancer risk have been suggested (Newmark and Lupton, 1990).

In this trial, faecal pH was measured directly using a solids pH probe. The measurement of faecal pH is non-invasive and represents differences in net excretion of acidic compounds between the two diets. The limitation of this is that net absorption of acidic and alkaline components produced by the microbiota along the GI tract, and the possible impact of transit time, renders faecal pH non-representative of the pH of luminal contents. While, direct measurement of luminal pH in the GI tract using radiotelemetric capsules or pH sensitive electrodes passed into the GI tract orally give an accurate measure of intraluminal pH, these methods are invasive, expensive and technically or logistically challenging.

4.5.4.2 pH measurement protocol

Stool samples were homogenised prior to direct measurement of stool pH. This was completed prior to any further stool processing.

The solids pH probe (InLab®, Mettler Toledo) was used for pH analysis. The probe was calibrated in stock pH 4.01 and 7.0 calibration buffer prior to each use. It was then immersed in the sample to a depth of approximately 1.5 cm or at least until the glass tip of the probe was fully immersed. The probe was held in the sample until a pH value was read and confirmed (FE20 FiveEasy™ Benchtop pH Meter).

4.6 Outcome measures and rationale: immunology

4.6.1 Biomarkers of intestinal inflammation

A thorough assessment of disease activity in IBD includes clinical, biochemical, endoscopic and radiologic measures (Peyrin-Biroulet et al.). Although disease activity indices such as the HBI include important GI symptoms for distinguishing active from inactive IBD, they do not correlate perfectly with endoscopic disease activity and symptoms may relate to non-inflammatory factors such as bile acid malabsorption (Yang et al., 2013b, Falvey et al., 2015). Furthermore, these measures are clearly of limited use as inclusion criteria in trials recruiting patients with suspected IBS-like symptoms, which are likely to elevate scores despite a lack of active inflammation. Endoscopy with histology is generally the preferred objective method for establishing disease activity (Magro et al., 2017, Gomollon et al., 2017). However, the invasive nature of these investigations in patients with inactive IBD would likely limit recruitment to a
clinical trial (Denters et al., 2013), particularly involving a dietary intervention not targeted at improving disease activity. Biomarkers, such as CRP, erythrocyte sedimentation rate, faecal calprotectin and faecal lactoferrin are non-invasive measures of inflammation and may be used as adjuncts to GI symptom assessment in the monitoring of IBD activity (Walsh et al., 2016). Some of these correlate with endoscopic and histologic IBD activity thus reducing unnecessary use of invasive, potentially unpleasant and costly investigations such as endoscopy and imaging (Schoepfer et al., 2013, Langhorst et al., 2008, Lehmann et al., 2015).

4.6.1.1 Faecal calprotectin

Calprotectin is a small, calcium-binding protein found in abundance in the cytoplasm of neutrophils and to a lesser extent, monocytes and macrophages (Steinbakk et al., 1990). During active IBD, neutrophils migrate from the circulation to the GI mucosa as part of the inflammatory cascade and are then shed into the lumen, where calprotectin is released due to cell disruption or death and can be measured in faeces (Brazil et al., 2013, Voganatsi et al., 2001). Faecal calprotectin has been used as a diagnostic aid in IBD (van Rheenen et al., 2010), to monitor GI inflammation in established IBD (D’Haens et al., 2012), as a predictor of mucosal healing (Røseth et al., 2004) and as a prognostic aid to predict IBD relapse (Mao et al., 2012). Faecal calprotectin is superior to serum CRP, blood leukocytes and clinical disease activity scores in differentiating endoscopically mild, moderate and severe disease in both CD and UC (Schoepfer et al., 2010, Schoepfer et al., 2013).

Appropriate faecal calprotectin cut-offs to define active and inactive IBD are controversial and ill-defined. Although a 250 µg/g cut-off predicted endoscopic IBD activity with optimal sensitivity and specificity (Lin et al., 2014), a more recent meta-analysis, published after the protocol for this study had been written, demonstrated a much lower 50 µg/g optimal cut-off through receiver operating characteristic curve analysis (Mosli et al., 2015). This discrepancy is likely partly explained by differences in study inclusion criteria. Although the use of a lower cut-off would increase sensitivity for detecting inactive IBD, this would result in exclusion of patients with inactive disease despite a slightly elevated faecal calprotectin. Therefore, patients had to have a screening faecal calprotectin of <250 µg/g to be enrolled.

Calprotectin concentrations in faeces can be quantified using ELISA or point-of-care (POC) test kits. There are a range of ELISA kits available, using different monoclonal and polyclonal antibodies to calprotectin (Ikhtaire et al., 2016). The CALPRO ELISA kit was shown to be superior to the EK-CAL and HK325 ELISA kits in identifying patients with IBD and in distinguishing between clinically active and inactive IBD (Mirsepas Lauridsen et al., 2016) and was chosen to quantify faecal calprotectin concentrations at baseline and end of trial.
4.6.1.1 Screening faecal calprotectin

Stool samples were collected following informed consent and faecal calprotectin was measured using the Bühlmann Quantum Blue® lower range quantitative immuno-chromatographic assay described in section 3.4.11.3, using an identical protocol. Patients with a screening faecal calprotectin of <250 µg/g were invited to complete a 7-day food and symptom diary as the second stage of screening.

4.6.1.2 Faecal calprotectin ELISA

Enzyme-linked immunosorbent assay (ELISA) allows quantification of peptides, proteins, antibodies and hormones. ELISA is based upon the immobilisation of an antigen of interest and detection through the assessment of enzyme activity, determined through colour changes. A standard curve is constructed from the absorbance of wells containing a standard concentration of the antigen of interest, thus allowing quantification of the substance in the test samples.

The calprotectin ELISA kit used in the current study (Calpro AS) uses a rabbit anti-calprotectin antibody and the enzyme is alkaline phosphatase.

4.6.1.3 Calprotectin extraction and ELISA protocol

At the baseline and end of trial visits, patients provided a whole stool sample to the investigator. An aliquot of 3-5g was reserved and frozen at -80°C for the ELISA.

Stool aliquots were thawed fully at room temperature prior to calprotectin extraction. Calprotectin extraction was carried out according to the manufacturer’s instructions and all reagents were provided with the kit unless otherwise stated (Calpro AS, Lysaker, Norway):

1. Faeces was loaded into the extraction device (Figure 4.3), avoiding trapping air bubbles and undigested solid materials. The sample was weighed (aiming for approximately 100 mg) and the exact weight documented.
2. The cap was fixed to the extraction device and extraction buffer was added in a 1:50 dilution.
3. The extraction device containing sample and buffer was vortexed for 3 minutes or until the sample was completely homogenised in the buffer, which was longer.
4. 500 µl of the faecal suspension was transferred to a sterile microtube and stored at 2-8°C until analysis the following day.
Calprotectin ELISA was carried out according to the manufacturer’s instructions and all reagents were provided with the kit unless otherwise stated (Calpro AS, Lysaker, Norway):

1. Faecal extracts were further diluted using the sample diluent solution. To ensure that all the test samples fell within the linear section of the standard curve, the dilution factor was adapted depending on the screening faecal calprotectin concentration for each patient. In patients whose screening faecal calprotectin of < 100 μg/g, a dilution of 1:25 was used. In patients with a screening faecal calprotectin of > 100 μg/g, a dilution of 1:50 was used.

2. Samples, standards, and the control solution were added to duplicate wells on the plate in 50 μl aliquots. Two wells were left blank, i.e. contained no test solution but received all other reagents for the ELISA. The baseline and end of trial samples for the same participant were included on the same plate, and each plate had a combination of CD and UC patients to avoid differences between these groups occurring as an artefact resulting from plate differences and variations in experimental conditions.

3. The plate was incubated at room temperature on a plate shaker for 45±5 minutes at 500-700 rpm, after which the wells were washed five times.

4. 50 μl of the enzyme conjugate was added to each well using a multi-channel pipette.

5. The plate was incubated at room temperature on a plate shaker for 45±5 minutes at 500-700 rpm and step 4 was repeated (five washes).
6. 100 μl of the enzyme substrate solution was added to each well. The plate was incubated for 30 minutes in a dark drawer at room temperature.

7. The optical density (OD) of the wells were read at 405 nm using a microplate reader.

8. The OD of the controls was plotted to create a standard curve. The optional highest standard (1000 μg/g) was not included in the ELISA since screening calprotectin had been performed 1-2 weeks prior to enrolment and had to be <250 μg/g for enrolment in the trial, therefore it was not expected that any samples would have a faecal calprotectin concentration greater than 250 μg/g. The highest included standard (500 μg/g) displayed very little difference in OD compared to the 250 μg/g standard for all three plates and was removed from the curve to retain the optimal fit of the line to the standards.

9. A logarithmic line was fitted as the standard curve, since this produced a line closest to all the standards. Therefore, a quadratic equation was used to calculate the calprotectin concentrations of the samples.

4.6.1.1.4 Advantages and limitations of ELISA

Enzyme-linked immunosorbent assay for faecal calprotectin permits a quantitative assessment of multiple samples at once and is therefore suited to batch sample analysis following a trial. It is the most commonly used method of faecal calprotectin quantification (Ikhtaire et al., 2016). However, despite being efficient and cost-effective batch sample analysis, ELISA is more labour intensive and requires greater laboratory expertise than POC test kits. Furthermore, samples with a higher calprotectin concentration can exceed the upper limit of the ELISA and the concentration of these samples cannot be calculated with accuracy. However, this was not an issue in this trial since the participants were recruited on the basis that their screening faecal calprotectin was <250 μg/g.

Calprotectin is resistant to heat and bacterial degradation, is stable in faeces for 3-7 days at room temperature (Røseth et al., 1992, Lasson et al., 2015) and has a homogeneous distribution in stool (Lasson et al., 2015). Excretion of faecal calprotectin in stools was shown to have low intra-individual variability on 3 consecutive days in a large cohort of patients with inactive CD (Naismith et al., 2013). Conversely, some studies have shown considerable day-to-day or within-day variation in faecal calprotectin, which may be more substantial in patients with IBD compared to healthy controls and may be greater in patients with active IBD and in patients with UC compared to CD (Husebye et al., 2001, Dobrzanski et al., 2014, Lasson et al., 2015, Naismith et al., 2013).
The rabbit-derived polyclonal anti-calprotectin antibody and the enzyme-conjugated antibody in the Calpro ELISA bind to several different epitopes on calprotectin, meaning the ELISA can detect calprotectin even if some epitopes are damaged or hidden due to complex formation with other components of stool.

4.6.1.2 C-reactive protein

C-reactive protein was measured in the current trial at screening and end of trial in the hospital pathology department. Active inflammation in IBD is associated with an acute phase reaction and therefore acute phase proteins such as CRP may be elevated in serum. Several studies have demonstrated that active IBD can be differentiated from functional bowel disorders based upon elevated serum CRP (Poullis et al., 2002, Shine et al., 1985). C-reactive protein may be used to predict endoscopic disease activity, albeit with only a modest correlation between them (Mosli et al., 2015). Blood tests are routinely performed in patients with IBD and the CRP assay is relatively cheap, making this an accessible method for monitoring IBD.

However, the correlation between serum CRP and endoscopic IBD activity is generally poorer than for faecal biomarkers such as faecal calprotectin and CRP may be a less sensitive marker of endoscopic activity in UC compared to CD (Lewis, 2011). Therefore, CRP in isolation is unlikely to be sufficient to define IBD activity but may be a useful adjunct to other biomarkers such as faecal calprotectin.

4.6.2 Peripheral T-cell gut-homing phenotype

Whole blood samples were collected by the author of this thesis at the baseline and end of trial. Whole-blood labelling and all flow cytometry analysis were performed on all samples by the author of this thesis at the Blizard Institute, Queen Mary University of London. Prior to the analysis of any participant samples, training in the required techniques and supervision of flow cytometry was provided by Dr Andrew Stagg (Reader in Immunology, Queen Mary University of London), Dr Neil McCarthy (Post-doctoral Research Fellow, Queen Mary University of London) and Inva Hoti (PhD student, Queen Mary University of London).

4.6.2.1 Principles of flow cytometry

Flow cytometry measures multiple physical and chemical characteristics of particles in a sample as they pass through one or several lasers and is used to investigate cell morphology and intracellular gene expression and to enumerate cell subsets. For example, it is commonly used to identify and quantify immune cell subsets within blood (Fischer et al., 2015, Globig et al., 2014, Hedin et al., 2014).
Flow cytometry measures size, granularity, internal complexity and emitted fluorescence of cells. To analyse cell subsets, fluorescent-labelled antibodies to cell surface markers specific to the subsets of interest are added and the cells are passed within a fluid in single-file through a laser intercept, allowing each cell to be assessed individually. Laser light hitting the cell is scattered and any fluorescent molecules present on the cell fluoresce. Via a collection filters, scattered and fluorescent light is directed to the appropriate wavelength-specific detectors, and finally electronic signals are produced. Different fluorophores are detected by different detectors, and can therefore be differentiated (Jahan-Tigh et al., 2012).

There is evidence of systemic inflammation in patients with IBD, for example serum cytokine concentrations may differ in IBD compared to healthy controls (Kleiner et al., 2015). Furthermore, serum concentrations of IL-6 and IL-8 been shown to change following a low FODMAP diet in IBS (Hustoft et al., 2017). However, serum cytokines may reflect inflammation outside the GI tract, and the aim of analysing immunology outcomes in this study was to establish whether the low FODMAP diet impacts upon GI inflammation specifically. The α4β7 integrin is a heterodimeric adhesion molecule expressed on the surface of leukocytes, facilitating entry of leukocytes into the intestine upon contact with mucosal addressin cell adhesion molecule-1 (MAdCAM-1) on venules of the mesenteric lymph nodes and Peyer’s patches (Berlin et al., 1993). Expression of α4β7 on circulating T-cells could therefore be considered a marker of their propensity to home to the GI tract and altered proportions of T-cell subsets expressing α4β7 have been observed in both CD and UC (Hedin et al., 2014, Fischer et al., 2015). Changes in the proportion of circulating T-cells expressing α4β7 could result from changes in GI inflammation, due to a greater number of T-cells primed to traffic to the GI tract, and therefore circulating T-cell phenotype could change according to disease activity. Therefore, flow cytometry was used to assess circulating T-cell phenotype as a proxy for GI immune function.

The whole blood labelling protocol was designed to permit enumeration of T-cell subsets, identified through the CD3 T-cell co-receptor. T-cells that have not yet been exposed to antigen are referred to as ‘naïve’ and express the longer CD45RA isoform. In contrast, effector/memory (or ‘antigen experienced’) T-cells typically express the shorter CD45RO isoform (Altin and Sloan, 1997). Therefore, CD45RA was used to identify naïve T-cells and effector/memory (cells negative for CD45RA).

Gut homing potential of T-cell subsets was assessed by labelling for α4β7. The beta subunit is also present at low concentrations in blood bound to αE (CD103). Labelling whole blood with an antibody to the β7 unit would detect both α4β7 and αEβ7, therefore antibodies specific to αE were included in the labelling panel to enable isolation of α4β7.
A small proportion express the γδ rather than the αβ T-cell receptor (typically <5%). These cells can rapidly expand during bacterial and parasitic infections and are activated by phosphoantigen released by the GI microbiota (Eberl et al., 2003). The majority of Vδ2 T-cells express α4β7 and promote inflammation in the GI mucosa (McCarthy et al., 2013), therefore an antibody to the Vδ2 complex was included in the labelling panel.

### 4.6.2.2 Antibodies

Anti-CD3 PB (clone SK7, Biolegend, San Diego, CA, USA), anti-CD8 PerCPCy5.5 (clone HIT8a, Biolegend), anti-CD45RA PE-Cy7 (clone HI100, Biolegend), anti-β7 PE (clone FIB504, Biolegend), anti-CD103 Alexafluor-647 (clone Ber-ACT8, Biolegend) and anti-Vδ2 FITC (clone B6, Biolegend) conjugated antibodies were used for the staining protocol (Table 4.1).

To determine gating for positive and negative populations of T-cell subsets, isotype-matched controls were used as follows: FITC mouse IgG1 (clone MOPC-21, Biolegend), PE rat IgG2a (clone RTK2758, Biolegend), PE-Cy7 mouse IgG1 (clone MOPC-21, Biolegend) and Alexafluor-647 mouse IgG1 (clone MOPC-21, Biolegend).

Single-colour controls were used to compensate for spectral overlap of fluorophores. Anti-CD8 FITC (clone SK1, Biolegend), anti-CD8 PE (clone SK1, Biolegend), anti-CD8 PerCPCy5.5 (clone HIT8a, Biolegend), anti-CD45RA PE-Cy7 (clone HI100, Biolegend), Anti-CD3 PB (clone SK7, Biolegend), and anti-CD8 Alexafluor-647 (clone SK1, Biolegend) were used as single-colour controls.

### 4.6.2.3 Sample collection and labelling

Blood samples were collected from participants at the baseline visit and the end of trial visit. Whole blood was collected into 4 ml lithium heparin vacutainers and stored at room temperature for labelling within 4 hours of collection.

Whole blood was antibody-labelled as follows:

1. Fluorescently labelled antibodies were added to 100μl whole blood according to the protocol in Table 4.1 and were incubated in the dark at room temperature for 15 minutes.

2. To each tube, 500 μl Optilyse C solution (Beckman Coulter, UK) was added to lyse red blood cells. These were vortexed and incubated in the dark at room temperature for 15 minutes.

3. Fluorescence activated cell sorting (FACS) buffer was added to each tube before centrifuging at 1700 rpm for 5 minutes.
4. The supernatant was discarded and cells were re-suspended in FACS buffer and centrifuged as per step 3.

5. The supernatant was discarded and the cells re-suspended in 300 μl 1% paraformaldehyde.

6. To the experimental tube only, 20 μl fluorescently-labelled beads (Flow-count fluorospheres, Beckman Coulter, UK) were added immediately prior to acquisition to determine absolute cell counts.

### Table 4.1 Protocol for antibody labelling of whole blood for flow cytometry

<table>
<thead>
<tr>
<th>Tube</th>
<th>FITC</th>
<th>PE</th>
<th>PerCPCy5.5</th>
<th>PE-Cy7</th>
<th>PB</th>
<th>AF647</th>
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<td><strong>Compensation tubes</strong></td>
<td></td>
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</tr>
<tr>
<td>1</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
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</tr>
<tr>
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<td></td>
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</tr>
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<td></td>
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<td><strong>Experimental tubes</strong></td>
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<td></td>
</tr>
<tr>
<td>8</td>
<td>Vδ2</td>
<td>B7</td>
<td>CD8</td>
<td>CD45RA</td>
<td>CD3</td>
<td>CD103</td>
</tr>
<tr>
<td>9</td>
<td>Vδ2</td>
<td>rlgG2a</td>
<td>CD8</td>
<td>CD45RA</td>
<td>CD3</td>
<td>CD103</td>
</tr>
<tr>
<td>10</td>
<td>mlgG1</td>
<td>B7</td>
<td>CD8</td>
<td>CD45RA</td>
<td>CD3</td>
<td>CD103</td>
</tr>
<tr>
<td>11</td>
<td>Vδ2</td>
<td>B7</td>
<td>CD8</td>
<td>CD45RA</td>
<td>CD3</td>
<td>mlgG1</td>
</tr>
<tr>
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<td>Vδ2</td>
<td>B7</td>
<td>CD8</td>
<td>CD45RA</td>
<td>CD3</td>
<td>CD103</td>
</tr>
</tbody>
</table>

FITC, Fluorescein isothiocyanate; PE, phycoerythrin; PerCPCy5.5, peridinin chlorophyll protein-cyanine 5.5; PE-Cy7, phycoerythrin-cyanine 7; PB, Pacific blue; AF647, alexafluor 647

**4.6.2.4 Data acquisition**

Following fixation of the cells, flow cytometry data was acquired using the BD FACSCanto II flow cytometer and the FACS DIVA software version 6.1.3 was used to collect data (BD Bioscience). The BD FACSCanto II contains the following lasers: blue (488-nm, air-cooled, 20-mW solid state), red (633-nm, 17-mW HeNe) and violet (405-nm, 30-mW solid state). For all compensation tubes and the blank tube, 20,000 events were recorded, while for the test and isotype control tubes 100,000 events were recorded.

**4.6.2.5 Data analysis**

Flow cytometry data was analysed contemporaneously using the Winlist software programme version 6.0 (Verity, Topsham, ME, USA).

First, compensation was performed for spectral overlap. Lymphocytes stained with single-colour monoclonal antibodies were used as the compensation standards (Table 4.1). A light scatter plot was constructed of forward scatter (relating to cell size) against side scatter (relating to cellular granularity) and a gate was drawn around the lymphocyte population (Figure 4.4).
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Figure 4.4 Compensation of spectral overlap. The left-hand histogram displays the fluorescence in the PE and PE-Cy7 channels for the PE single-colour control. There is fluorescence in the PE-Cy7 channel despite a lack of PE-Cy7 staining in the tube. Compensation assesses the geometric mean of the fluorescence in the y-axis and adjusts fluorescence to eliminate this overlap (right-hand histogram).

The degree of overlapping fluorescence between fluorophores was assessed by constructing two-parameter plots containing each possible permutation of the fluorophores used in the protocol (Table 4.1) and then activating the software-generated compensation (Figure 4.4). Plots were visually inspected and any remaining overlap was manually adjusted by assessing and adjusting the geometric mean of fluorescence on the y axis of the two-parameter plot. The compensation protocol was saved once all fluorophores had been compensated.
4.6.2.6 Analysis of flow cytometry data

The file corresponding to the experimental tubes was analysed with the compensation protocol applied. Again, using a light scatter plot, lymphocytes were gated (Figure 4.7).

![Figure 4.5 Gating of the CD45RA-positive population with reference to the isotype control. In the left-hand plot, CD4+ T-cells were stained with PE-Cy7 conjugated anti-mlgG1 antibodies (isotype control). The CD45RA-positive and negative populations were then defined with reference to the peak of this isotype control (with the positive population presumably lying to the right of isotype control peak). The right-hand plot shows the same gates applied to the test tube file (containing CD4+ T-cells stained with PE-Cy7 conjugated anti-CD45RA antibodies).](image-url)

As isotype-matched control antibodies were not included for CD3 and CD8, these populations were gated by eye. For all other populations, the isotype-matched control tube was used to set the gating for the positive and negative populations, as illustrated in Figures 4.5 and 4.6.

Within the analysis protocol, the flow count beads added to the experimental tube were identified by gating the highest peak on an ungated, one-parameter FITC plot. Absolute cell counts for each population of interest were calculated using the following equation:

\[
N = 1000 c \left( \frac{r}{b} \right)
\]

Where:

- \(N\) = number of events per ml of blood analysed
- \(c\) = concentration of beads in the bead solution (beads/\(\mu\)l)
- \(r\) = number of events in the gate of interest
- \(b\) = number of events in the bead gate

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4.6.2.7 Flow cytometry gating strategies to investigate α4β7 expression on T-cell subsets

The same gating strategy for enumerating α4β7+ naïve and effector/memory CD4+ and CD8+ T-cells was used for all samples in the trial and is illustrated for the CD4+ population in Figure 4.7. From the lymphocyte population (derived from a light scatter plot), the Vδ2 T-cell population were identified by plotting CD3-PB against Vδ2-FITC. The population positive for only CD3-PB was assumed to contain CD4+ and CD8+ T-cells. A one-parameter histogram of CD8-PerCPCy5.5 was then plotted and the negative population was assumed to be CD4+ T-cells. Within the CD4+ and CD8+ populations, a one-parameter plot of CD45RA-PE-Cy7 was then used to determine the naïve and effector/memory populations using the PE-Cy7-conjugated anti-mIgG1 isotype control. Two-parameter plots of β7 against CD103 were constructed for the naïve and effector/memory CD4+ and CD8+ T-cells. Using the PE-conjugated anti-rIgG2a and AF647-conjugated anti-mIgG1 isotype controls, the populations positive for only β7 were assumed to be the α4β7-positive populations, while the populations positive for both PE conjugated anti-β7 and AF647 conjugated anti-CD103 antibodies were assumed to be αEβ7-positive T-cells (Figure 4.7). A one-parameter plot of the Vδ2 T-cells was constructed to determine the proportion expressing α4β7.

Figure 4.6 Gating strategy for defining α4β7-positive αEβ7-negative populations. The left panel shows the CD4+ T-cells stained with PE conjugated anti-rIgG2a antibodies (isotype control). The vertical cross-hair was set to define the PE positive and negative populations. The middle plot shows the CD4+ T-cells stained with AF647 conjugated anti-mIgG1 antibodies (isotype control). The horizontal cross-hair was set to define the AF647 positive and negative populations. The right-hand plot shows the same gates applied to the test tube file (containing CD4+ T-cells stained with PE conjugated anti-β7 and AF647 conjugated anti-CD103 antibodies).
Figure 4.7 Strategy used to determine α4β7-positive populations within naïve and memory CD4+ and CD8+ T-cell populations. A light scatter plot was constructed and lymphocytes gated. From this "live" gate, a two-parameter plot of CD3 against delta-2 was constructed. The ‘double-positive’ population was gated, assumed to be the \( V_\delta^2 \) T-cell population (R2) and the single-positive CD3+ population was gated (containing the CD4+ and CD8+ T-cells; R3). From the single-positive CD3+ population, a one-parameter plot of CD8 was plotted. The negative population was assumed to represent the CD4+ T-cell population. From the CD8+ and CD4+ T-cell populations (R4 and R5), a one-parameter plot of CD45RA was used to define the naïve (CD45RA+) and effector/memory (CD45RA-) populations (R8 and R9 display this for CD4+ T-cells only). From the naïve and memory populations, two-parameter plots of \( \beta_7 \) vs. CD103 were used to define the α4β7-positive populations (R18 and R22 show the α4β7-positive naïve and memory CD4+ T-cell population) with reference to the isotype control, Figure 3.4.

The number of events in each gated region was used to determine the proportions and absolute numbers of T-cell subsets. For example, to calculate the proportion of naïve CD4+ T-cells expressing α4β7, the number of events in that region was divided by the number of events in the naïve CD4+ T-cell region and multiplied by 100.
4.6.2.8 Advantages and limitations of flow cytometry

Flow cytometry is a highly efficient technique allowing identification and characterisation of up to 20 parameters of cell phenotype simultaneously. It is highly specific and can be used to identify rare cells within a sample since each cell is examined individually (Jahan-Tigh et al., 2012). This contrasts with transcriptional profiling, immunoblotting and PCR that provide no information on individual cells. In this trial, flow cytometry permitted the investigation of peripheral T-cell subsets without the need for the more laborious and technical methods required to extract T-cells from other tissues.

The focus of the flow cytometry experiments in this study was the expression of $\alpha_4\beta_7$ on T-cell subsets. The labelling protocol allowed differentiation between $\alpha_4\beta_7$ and $\alpha E\beta_7$-positive T-cells, thus being more specific for $\alpha_4\beta_7$-positive T-cells.

There remains a lack of standardisation in flow cytometry experiments and analysis, limiting comparisons of results between studies. Additionally, gating relies purely on the person performing the analysis and is therefore subject to inter-observer bias. However, all analysis was performed by one investigator (SC) in this study and therefore gating was consistent throughout.

There is some debate over the specificity of some markers to detect cell subsets. Naïve T-cells are typically identified through CD45RA expression, however CD45RA can also be expressed by senescent and terminally differentiated CD8 effector T-cells (Henson et al., 2014, Hoflich et al., 1998), and can be re-expressed by CD4 memory T-cells (Arlettaz et al., 1999). Therefore, a small proportion of CD45RA+ T-cells may not be truly naïve.
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4.7 Outcome measures and rationale: Nutrient intake

4.8 Food diaries, diary administration, data input and analysis

Prospective 7-day unweighed food diaries were completed prior to enrolment and in the final week of the diet to examine energy, nutrient and fermentable carbohydrate intakes. The rationale for this method of dietary assessment is described in section 2.4.8.1. The diaries were identical to those used in the case-control study in Chapter 2 and were administered in an identical fashion (section 2.4.8.2). Food diaries were checked for completeness at the baseline and end of trial visits and additional information was requested if required. All food diaries were entered into Nutritics by Charlotte Howard (student dietitian, King’s College London) using the same protocol described in section 2.4.8.1. Data were analysed by the author of this thesis (SC) as described in section 2.4.8.5.

4.8.1.1 FODMAP intake analysis

Food diary data were analysed for FODMAP intakes at baseline and end of trial by the author of this thesis using identical methods to those described in section 2.4.8.4.

4.8.2 Food-related quality of life

The rationale for measuring FR-QOL has been discussed in section 2.4.9.2. The same validated questionnaire was used to assess FR-QOL at baseline and end of trial in this trial as that described in section 2.4.9.2.

4.9 Outcome measures and rationale: diet acceptability

Patient acceptability of an intervention is likely determined by factors such as like or dislike for the treatment modality, perceived benefit of the treatment and a lack of knowledge, at least in IBS (Harris and Roberts, 2008). Acceptability clearly has the potential to impact upon compliance and is therefore an important outcome in trials of treatments that could undergo widespread dissemination and use in clinical practice.

Acceptability questionnaires for dietary interventions assess factors such as palatability, ease of food purchase and preparation, effort required to follow the diet, convenience of the diet and satisfaction with meals during the diet (Barnard et al., 2009, Moore et al., 2015)

There are no validated acceptability questionnaires for the low FODMAP diet. However, a previous PhD student developed a questionnaire for measuring acceptability of the low FODMAP diet in a trial of patients with IBS with a similar design to the current trial. The answers were based on a Likert scale and included questions regarding palatability, ease of meal
preparation and time spent shopping and cooking, ease of locating suitable foods, cost and convenience of the diet. This questionnaire was also used to assess long-term acceptability of the low FODMAP diet in IBS (O’Keeffe et al., 2018).

4.10 Statistical analysis

Data collection, entry and statistical analysis, with the exception of microbiome data, was conducted by the author of this thesis with guidance from the Statistical Consultancy Service at King’s College London. Statistical analysis was performed after the last participant’s end of trial data had been collected, using IBM SPSS Statistics for Windows Version 24.0.

Prior to running statistical tests, continuous data were explored for normality via visual inspection of histograms. Demographic data were analysed between the diet groups at baseline using an independent t-test for continuous variables and a Chi-squared test for categorical variables.

Normally-distributed continuous variables were compared between groups at baseline using independent t-tests. End of trial values were compared using analysis of covariance (ANCOVA) with the baseline value as a covariate. Non-normally distributed variables were transformed prior to performing ANCOVA. Categorical variables were compared using the Chi-squared test or Fisher’s Exact Test where expected counts in contingency tables were below five.

Logistic regression was used to determine the degree to which diet allocation predicted categorical variables and multiple linear regression was used to investigate the degree to which diet allocation predicted continuous variables. Pearson or Spearman correlation analyses were used to investigate correlations between continuous variables.

Metagenomic bioinformatics analysis was performed by Sébastien Fromentin (Bio-analyst, INRA). Microbiome data were compared between diet groups at end of trial (accounting for baseline microbial abundance) and between baseline and end of trial in each of the diet groups using a likelihood ratio test. This test compares the goodness of fit of two nested statistical models; the null model and the alternative model. If the value of the likelihood ratio is very small, then it is likely to reach statistical significance, thus concluding a difference between the compared data sets.

Data are presented as mean (SD), plus mean difference and 95% confidence intervals (CI’s). Categorical variables are presented as number (%). Data were considered significant when $P \leq 0.05$. 

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4.10.1 Analysis sets
The intention to treat (ITT) dataset included all participants enrolled in the trial, regardless of compliance to the intervention and whether the trial was completed. Intention to treat analysis provides a more conservative estimate of the effect size of an intervention and represents the likely effectiveness in clinical practice, where patients may not complete or adhere to prescribed interventions. This eliminates bias that could be introduced through excluding participants who may systematically differ from those completing the trial, thus to some extent nullifying randomisation (Ranganathan et al., 2016, Gupta, 2011). The Rome committee recommends the use of ITT analysis, however acknowledging that per protocol (PP) analysis has its place for demonstrating the efficacy of the treatment under optimal conditions (Irvine et al., 2016). Furthermore, similar results in the ITT and PP analysis provides further confidence in the findings. A PP analysis, excluding participants who withdrew from the trial prematurely along with those failing to comply with the allocated diet ≥75% of the time, was therefore also conducted for all outcomes in this trial.

4.10.2 Missing and ambiguous data
To avoid missing data, all questionnaires and diaries were checked by the author of this thesis during trial visits. Participants were then encouraged to complete any missing fields.

There are several methods for handling missing data, including modelling techniques and simple and multiple imputation methods (Powney et al., 2014). Multiple imputation accounts for uncertainty regarding missing data by creating a combination of different plausible imputed data sets (Sterne et al., 2009). This reduces bias associated with the assumption that outcomes were the same at baseline and at the point of withdrawal from the trial for patients who were prematurely withdrawn.

However, multiple imputation is most effective where there are reliable predictors of outcome (Vickers and Altman, 2013). In this trial it was not expected that baseline values would correlate with values throughout the trial, therefore multiple imputation was not appropriate. Missing data was instead imputed using Last Observation Carried Forward to allow an ITT analysis.
5 Results: the effect of fermentable carbohydrate restriction on clinical and diet outcomes in patients with inactive inflammatory bowel disease
5.1 Introduction

This thesis has thus far demonstrated that a proportion of patients with IBD restrict dietary FODMAP intake (Chapter 2), and that FODMAPs, particularly fructans, induce functional gastrointestinal symptoms (FGS) in patients with inactive IBD on the background of a low FODMAP diet (Chapter 3). Although Chapter 3 demonstrated that a pure fructan challenge induces FGS, it cannot confirm that the low FODMAP diet (restricting the dietary intake of several fermentable carbohydrates) is effective for reducing FGS in patients with inactive IBD.

A recent randomised, placebo-controlled trial indicated that a low FODMAP diet is effective for the management of gastrointestinal (GI) symptoms in IBS (Staudacher et al., 2017b). However, to date, evidence of the clinical effectiveness of the diet in patients with inactive IBD with FGS is limited to retrospective and prospective uncontrolled studies and a non-blinded RCT (Prince et al., 2016, Gearry et al., 2009b, Pedersen et al., 2017). In the non-blinded RCT, significantly more patients in the low FODMAP diet group responded to the diet compared to patients continuing their habitual diets (Pedersen et al., 2017). However, 46% of participants in the habitual diet group were also classified as responders, representing a significant placebo response as a result of simply taking part in research (Sedgwick and Greenwood, 2015). This reinforces the need for a placebo-controlled trial of the low FODMAP diet in IBD.

Therefore, a RCT was designed to investigate the effect of low FODMAP dietary advice compared to placebo ‘sham’ dietary advice on FGS, the GI microbiome and markers of intestinal inflammation in patients with inactive IBD, the methods for which are described in Chapter 4.

This chapter reports the effects of the low FODMAP diet on GI symptoms, stool output, clinical disease activity, health-related quality of life (HR-QOL), food-related quality of life (FR-QOL), patient-perceived control of IBD, nutrient and FODMAP intakes, and diet acceptability. CONSORT guidelines were followed in reporting outcomes of this RCT (Schulz et al., 2010).

5.2 Patient recruitment and progress, characteristics, compliance and adverse events

5.2.1 Patient recruitment and progress

Trial screening began in December 2015, the first participant was recruited on 25th February 2016 and the last participant completed the trial on 16th May 2017. Figure 5.1 shows the CONSORT diagram for the trial.

Of 155 patients screened, 98 were excluded during initial screening because they met exclusion criteria (Figure 5.1). Following completion of the 7-day baseline food and symptom diary, five patients were excluded due to not experiencing symptoms with sufficient incidence and severity
(at least one of: abdominal pain, bloating or diarrhoea at least two days per week during the screening week) (section 4.2.1.3). Therefore, a total of 52 patients were enrolled in the trial, 27 to the low FODMAP diet and 25 to the sham diet. Twenty-three participants were recruited from Guy’s and St Thomas’ NHS Foundation Trust and 29 from Barts Health NHS Trust.

Forty-six patients completed the trial and six were withdrawn; two withdrew consent during the trial (one in each group), one became pregnant (sham diet group), two commenced steroids due to the onset of symptoms of active IBD (one in each group), and one commenced antibiotics for an infection unrelated to IBD (low FODMAP diet group). Of the 46 patients completing the trial, three were deemed non-compliant to the diet (defined as following the diet ≤75% of the time overall during the trial, assessed at the end of trial visit) and were excluded from the per protocol (PP) analysis, therefore leaving 43 participants (21 in the low FODMAP diet group and 22 in the sham diet group).
Figure 5.1 CONSORT diagram depicting participant flow through the RCT. FC, faecal calprotectin; CRP, C-reactive protein.
5.2.2 Baseline patient demographic and clinical characteristics

Baseline demographic data are presented in Table 5.1. Patients in the low FODMAP diet group were significantly younger than those in the sham diet group (33 SD 11 years vs. 40 SD 13 years, P=0.031). Furthermore, significantly more patients in the low FODMAP diet group were white compared to the sham diet group (25/27, 93% vs. 19/25, 76%, P=0.029). There were no significant differences in any other baseline demographics between the groups.

Table 5.1 Baseline demographic characteristics of the ITT population (n=52)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Low FODMAP diet (n=27)</th>
<th>Sham diet (n=25)</th>
<th>P</th>
</tr>
</thead>
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<tr>
<td>Age (years)</td>
<td>33 (11)</td>
<td>40 (13)</td>
<td>0.031</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>10 (37)</td>
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</tr>
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<td>Current smokers, n (%)</td>
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</tr>
<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24 (3)</td>
<td>25 (4)</td>
<td>0.526</td>
</tr>
<tr>
<td>Ethnicity, white, n (%)</td>
<td>25 (92)</td>
<td>19 (76)</td>
<td>0.029</td>
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<tr>
<td>Rome III criteria, n (%)</td>
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<tr>
<td>IBS-D</td>
<td>10 (37)</td>
<td>5 (20)</td>
<td>0.150</td>
</tr>
<tr>
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<td>2 (7)</td>
<td>2 (8)</td>
<td></td>
</tr>
<tr>
<td>IBS-U</td>
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</tr>
<tr>
<td>Functional bloating</td>
<td>15 (56)</td>
<td>13 (52)</td>
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</tr>
<tr>
<td>Functional diarrhoea</td>
<td>0 (0)</td>
<td>4 (16)</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean (SD) unless otherwise stated.

BMI, body mass index; IBS-D, IBS diarrhoea subtype; IBS-M, IBS mixed subtype; IBS-U, IBS unsubtyped

Baseline clinical variables relating to IBD are displayed in Table 5.2. Twenty-six participants each with CD and UC were recruited. There were no significant differences in any clinical characteristics between the diet groups.

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### Table 5.2 Baseline IBD characteristics of the ITT population (n=52)

<table>
<thead>
<tr>
<th></th>
<th>Low FODMAP diet (n=27)</th>
<th>Sham diet (n=25)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Crohn’s disease, n (%)</strong></td>
<td>14 (52)</td>
<td>12 (48)</td>
<td>0.781</td>
</tr>
<tr>
<td><strong>Time since diagnosis, years</strong></td>
<td>7 (8)</td>
<td>11 (11)</td>
<td>0.187</td>
</tr>
<tr>
<td><strong>Time since last flare, years</strong></td>
<td>2 (1)</td>
<td>4 (8)</td>
<td>0.193</td>
</tr>
<tr>
<td><strong>Montreal classification</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crohn’s disease location, n (% of CD)</td>
<td></td>
<td></td>
<td>0.773</td>
</tr>
<tr>
<td>Ileal</td>
<td>4 (29)</td>
<td>2 (17)</td>
<td></td>
</tr>
<tr>
<td>Colonic</td>
<td>4 (29)</td>
<td>4 (33)</td>
<td></td>
</tr>
<tr>
<td>Ileocolonic</td>
<td>6 (42)</td>
<td>6 (50)</td>
<td></td>
</tr>
<tr>
<td>Crohn’s disease behaviour, n (% of CD)</td>
<td></td>
<td></td>
<td>0.949</td>
</tr>
<tr>
<td>Non-stricturing, non-penetrating</td>
<td>9 (64)</td>
<td>8 (66)</td>
<td></td>
</tr>
<tr>
<td>Stricturing</td>
<td>3 (21)</td>
<td>2 (17)</td>
<td></td>
</tr>
<tr>
<td>Penetrating</td>
<td>2 (14)</td>
<td>2 (17)</td>
<td></td>
</tr>
<tr>
<td>Perianal disease, n (% of CD)</td>
<td>4 (29)</td>
<td>3 (25)</td>
<td>1.000</td>
</tr>
<tr>
<td>Ulcerative colitis extent, n (% of UC)</td>
<td></td>
<td></td>
<td>0.403</td>
</tr>
<tr>
<td>Proctitis</td>
<td>6 (46)</td>
<td>3 (23)</td>
<td></td>
</tr>
<tr>
<td>Left-sided</td>
<td>4 (31)</td>
<td>7 (54)</td>
<td></td>
</tr>
<tr>
<td>Extensive</td>
<td>3 (23)</td>
<td>3 (23)</td>
<td></td>
</tr>
<tr>
<td><strong>Clinical disease activity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crohn’s disease (HBI)</td>
<td>4 (2)</td>
<td>5 (2)</td>
<td>0.427</td>
</tr>
<tr>
<td>Ulcerative colitis (Partial Mayo)</td>
<td>0.8 (0.9)</td>
<td>0.4 (0.7)</td>
<td>0.233</td>
</tr>
<tr>
<td><strong>Surgery, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small intestinal resection</td>
<td>1 (4)</td>
<td>1 (4)</td>
<td>1.000</td>
</tr>
<tr>
<td>Right hemicolectomy</td>
<td>3 (12)</td>
<td>2 (7)</td>
<td>0.662</td>
</tr>
<tr>
<td><strong>Medication, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-ASA</td>
<td>12 (44)</td>
<td>11 (44)</td>
<td>0.974</td>
</tr>
<tr>
<td>Thiopurine</td>
<td>9 (33)</td>
<td>12 (48)</td>
<td>0.282</td>
</tr>
<tr>
<td>Infliximab</td>
<td>10 (37)</td>
<td>4 (16)</td>
<td>0.087</td>
</tr>
<tr>
<td>Adalimumab</td>
<td>2 (7)</td>
<td>4 (16)</td>
<td>0.411</td>
</tr>
<tr>
<td>Vedolizumab</td>
<td>0 (0)</td>
<td>1 (4)</td>
<td>0.481</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>2 (7)</td>
<td>1 (4)</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Values are mean (SD) unless otherwise stated.
HBI, Harvey-Bradshaw Index

### 5.2.3 Compliance

During weekly telephone calls with the researcher, participants were asked about their diet adherence over the preceding week. Most of the participants (42/52, 81%) reported following the diet >75% of the time on all three phone calls for both diets. When asked at the end of trial visit about overall adherence during the entire four-week trial, 24/27 (88%) of participants in the low FODMAP diet group and 25/25 (100%) in the sham diet group reported following the diet >75% of the time (P=0.230). Of the remaining patients in the low FODMAP diet group, 2/27 (8%) reported following the diet 50-75% of the time and 1/27 (4%) reported following the diet 25-50% of the time overall. There was no significant difference in the proportion of patients with CD and UC adhering to the diet >75% of the time (24/26, 91% CD vs. 25/26, 96% UC, P=0.570).
5.2.4 Adverse events

There were six adverse events during the trial. Two participants had an IBD relapse (one with CD and one with UC; one in the low FODMAP group and one in sham group), both requiring a course of steroids. One participant in the low FODMAP diet group commenced antibiotics for a nasolabial cyst, unrelated to the diet. All three participants were withdrawn from the trial due to meeting exclusion criteria. One participant in the sham diet group reported a worsening of abdominal pain in the second week of the trial lasting two days. Upon discussion with the referring consultant, it was not thought to relate to the diet and the participant completed the trial. One participant in the low FODMAP diet group reported flu-like symptoms and one in the sham diet group experienced sinusitis, both of which were unrelated to the diet and the patients completed the trial. All patients were included in the ITT analysis.
5.3 Gastrointestinal symptoms and stool output

5.3.1.1 IBS-SSS

5.3.1.1.1 All participants

The IBS Severity Scoring System (IBS-SSS) outcomes at end of trial for the ITT and PP populations are presented in Table 5.3. There was no significant difference in the primary outcome (change in IBS-SSS score between baseline and end of trial) between the low FODMAP diet (-67, SD 78) and the sham diet group (-34, SD 50) (P=0.075) (also see Figure 5.2).

In terms of other IBS-SSS outcomes, there was no difference between the diet groups in the total end of trial score or in any of the subscores except for bloating severity (question 2), which was significantly lower in the low FODMAP diet (23 SD 16) than the sham diet group (34 SD 15) at end of trial (P=0.021) (Table 5.3). The PP analysis showed similar results to the ITT analysis for the end of trial IBS-SSS score and subscores (Table 5.3). Linear regression showed diet allocation and diagnosis were not associated with end of trial IBS-SSS score $F(2, 49)=1.82$ (P=0.173). There was no difference between the groups in the proportion of patients reaching the MCID of a 50-point reduction in IBS-SSS score between baseline and end of trial. However, significantly more participants achieved a 50% reduction in total IBS-SSS score between baseline and end of trial in the low FODMAP diet (9/27, 33%) than in the sham diet group (1/25, 4%; P=0.012) (Table 5.3).
Table 5.3 IBS-SSS change, total score, sub-scores and proportion of patients achieving the minimally clinically important difference at end of trial

<table>
<thead>
<tr>
<th></th>
<th>Intention to treat analysis</th>
<th>Per protocol analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low FODMAP diet</td>
<td>Sham diet</td>
</tr>
<tr>
<td></td>
<td>(n=27)</td>
<td>(n=25)</td>
</tr>
<tr>
<td>Change in IBS-SSS score</td>
<td>-67 (78)</td>
<td>-34 (50)</td>
</tr>
<tr>
<td>Total IBS-SSS score</td>
<td>157 (68)</td>
<td>190 (65)</td>
</tr>
<tr>
<td>Pain severity</td>
<td>22 (16)</td>
<td>30 (15)</td>
</tr>
<tr>
<td>Days of pain (days)</td>
<td>36 (26)</td>
<td>38 (25)</td>
</tr>
<tr>
<td>Bloating severity</td>
<td>23 (16)</td>
<td>34 (15)</td>
</tr>
<tr>
<td>Satisfaction with bowels</td>
<td>39 (16)</td>
<td>47 (20)</td>
</tr>
<tr>
<td>Impact on life</td>
<td>38 (16)</td>
<td>41 (15)</td>
</tr>
<tr>
<td>IBS-SSS 50-point reduction(a), n (%)</td>
<td>15 (56)</td>
<td>9 (36)</td>
</tr>
<tr>
<td>IBS-SSS 50-percent reduction(b), n (%)</td>
<td>9 (33)</td>
<td>1 (4)</td>
</tr>
</tbody>
</table>

All values are mean (SD) adjusted for baseline values using ANCOVA, unless otherwise stated. Estimated mean differences and 95% CI’s are presented for continuous outcomes. IBS-SSS, Irritable bowel syndrome severity scoring system.

\(a\) Patients achieving at least a 50-point reduction and \(b\) a 50-percent reduction in total IBS-SSS score between baseline and end of trial.
5.3.1.1.2 Crohn’s disease and ulcerative colitis

Sub-group analyses of the primary outcome were performed in CD and UC. In patients with CD (n=26), there was no difference in the change in IBS-SSS score during the trial between the low FODMAP diet (-60 SD 90) and sham diet group (-39 SD 52) (P=0.515) (Figure 5.2). There was no significant difference in the total end of trial IBS-SSS score between the diets or any of the IBS-SSS sub-scores between the diets (data not shown). There was also no difference between the low FODMAP and sham diet groups in the proportion of patients reaching the MCID (50-point reduction) (7/14, 50% vs. 5/12, 42%, P=0.671) or a 50% reduction in score in the IBS-SSS score (5/14, 36% vs. 1/12, 8%, P=0.170).

In patients with UC (n=26), there was a significantly greater reduction in IBS-SSS score between baseline and end of trial in the low FODMAP diet (-77 SD 54) compared with the sham diet group (-29 SD 50) (P=0.031) (Figure 5.2), in addition to a significantly lower total IBS-SSS score at end of trial (135 SD 69 vs. 184 SD 69, P=0.031). There was no significant difference in any of the IBS-SSS sub-scores between the diet groups (data not shown) or in the proportion of patients reaching the MCID (50-point reduction) (8/13, 62% vs. 4/13, 31%, P=0.116) or a 50% reduction in the IBS-SSS score (4/13, 31% vs. 0/13, 0%, P=0.096).

Considering that a greater reduction in IBS-SSS during the trial was observed in patients with UC following the low FODMAP diet, a linear regression analysis was performed with the change in

Figure 5.2 Change in IBS-SSS score between baseline and end of trial in all participants (n=52) (left panel), and patients with CD (n=26) (middle panel) and UC (n=26) (right panel). Values are mean (SEM) adjusted for baseline using ANCOVA. IBS-SSS, irritable bowel syndrome severity scoring system
IBS-SSS score between baseline and end of trial as the dependent variable and diet allocation and diagnosis as independent variables. The regression model was not associated with change in IBS-SSS score \( F(2, 49) = 1.49 \) (\( P=0.236 \)).

### 5.3.1.1.3 Factors associated with response to the low FODMAP diet

Given that the mechanisms of a low FODMAP diet on GI symptoms are largely hypothesised to involve bacterial fermentation and colonic gas production, it was important to determine the effect of the diet on patients with and without colonic disease. There were six patients in the trial with purely ileal CD (i.e. lacking colonic disease); four in the low FODMAP diet group and two in the sham diet group. In a sensitivity analysis excluding these participants, the reduction in IBS-SSS score during the trial became significantly greater in the low FODMAP diet group (-76 SD 68) compared to the sham diet group (-36 SD 46) (\( P=0.033 \)). Furthermore, ANCOVA was performed with all participants included but controlling for the presence of colonic disease as a covariate. Here, the difference between the groups failed to reach significance (-66 SD 81 vs. -35 SD 46, \( P=0.050 \)).

In the only other previous RCT of low FODMAP dietary advice in IBD, the diet appeared to be less efficacious in patients with high faecal calprotectin at baseline (Pedersen et al., 2017). Therefore, a possible correlation between baseline faecal calprotectin with change in IBS-SSS score during the trial was examined. This showed a moderate positive correlation, suggesting a lower baseline faecal calprotectin to be associated with a greater decrease in IBS-SSS score during the trial (\( r=0.425, P=0.002 \)). It was not possible to perform sub-group analysis according to baseline faecal calprotectin since only 9 patients had faecal calprotectin above 100 \( \mu g/g \).

### 5.3.1.2 Adequate relief

Significantly more participants reported adequate relief of GI symptoms at end of trial in the low FODMAP diet (14/27, 52%) compared with the sham diet group (4/25, 16%) (\( P=0.007 \)) in the ITT population (Figure 5.3). Likewise, significantly more patients reported adequate relief at end of trial in the low FODMAP diet (13/21, 62%) compared with the sham diet group (4/22, 18%) (\( P=0.003 \)) in the PP population.
There was no difference in the proportion of participants reporting adequate relief at end of trial between the low FODMAP diet and sham diet groups in patients with CD (7/14 vs. 2/12, P=0.110) or UC (7/13 vs. 2/13, P=0.097).

A logistic regression analysis including all participants revealed that a model including diet allocation and diagnosis as independent variables was significantly associated with the likelihood of reporting adequate relief $\chi^2(2)=7.72$ (P=0.021). The model predicted 19% of the variance in adequate relief and correctly classified 67% of cases. The odds of reporting adequate relief were 5.67 times greater in the low FODMAP diet than the sham diet group (95% CI 1.53, 20.93; P=0.009), but diagnosis was not significantly associated with reporting adequate relief (OR 1.07, 95% CI 0.31, 3.69; P=0.910).
5.3.1.3 **Gastrointestinal Symptom Rating Scale**

The end of trial incidence (number of days on which each symptom was reported during the final week of the diet) and severity (7-day average symptom severity) of 15 individual symptoms and overall symptoms on the Gastrointestinal Symptom Rating Scale (GSRS) are reported for the ITT population in Table 5.4. There were no differences in the end of trial incidence or severity of any of the symptoms assessed, except for lower severity of flatulence in the low FODMAP diet (0.9 SD 0.5) compared to the sham diet group (1.2 SD 0.5) (P=0.035). Analysis of the PP population also revealed no differences in the incidence of moderate or severe symptoms between groups, or the severity of any symptoms except for a significantly lower severity of flatulence at end of trial in the low FODMAP diet (0.8 SD 0.4) compared to the sham diet group (1.1 SD 0.4) (P=0.028). There was no difference in the incidence or severity of any GSRS symptoms between the diet groups at end of trial in CD or UC (data not shown).
### Table 5.4 Incidence and severity of GI symptoms as measured by the Gastrointestinal Symptom Rating Scale at end of trial in the ITT population (n=52)

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Incidence(^a) of moderate or severe symptoms</th>
<th>Severity(^b) of symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low FODMAP diet (n=27)</td>
<td>Sham diet (n=25)</td>
</tr>
<tr>
<td>Pain</td>
<td>1.5 (1.3)</td>
<td>1.1 (1.3)</td>
</tr>
<tr>
<td>Heartburn</td>
<td>0.3 (0.5)</td>
<td>0.2 (0.5)</td>
</tr>
<tr>
<td>Acid regurgitation</td>
<td>0.3 (0.5)</td>
<td>0.2 (0.5)</td>
</tr>
<tr>
<td>Nausea</td>
<td>0.5 (0.6)</td>
<td>0.3 (0.6)</td>
</tr>
<tr>
<td>Gurgling</td>
<td>0.7 (1.0)</td>
<td>0.8 (1.0)</td>
</tr>
<tr>
<td>Bloating</td>
<td>1.4 (1.7)</td>
<td>1.7 (1.7)</td>
</tr>
<tr>
<td>Belching</td>
<td>0.2 (0.6)</td>
<td>0.5 (0.6)</td>
</tr>
<tr>
<td>Flatulence</td>
<td>1.4 (1.8)</td>
<td>2.1 (1.8)</td>
</tr>
<tr>
<td>Constipation</td>
<td>0.5 (1.1)</td>
<td>0.6 (1.1)</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>0.4 (0.7)</td>
<td>0.5 (0.7)</td>
</tr>
<tr>
<td>Loose stools</td>
<td>0.9 (1.0)</td>
<td>0.9 (1.0)</td>
</tr>
<tr>
<td>Hard stools</td>
<td>0.1 (0.7)</td>
<td>0.3 (0.7)</td>
</tr>
<tr>
<td>Urgency</td>
<td>0.9 (1.1)</td>
<td>0.8 (1.1)</td>
</tr>
<tr>
<td>Incomplete evacuation</td>
<td>0.7 (1.2)</td>
<td>0.5 (1.2)</td>
</tr>
<tr>
<td>Tiredness</td>
<td>2.3 (1.8)</td>
<td>2.1 (1.8)</td>
</tr>
<tr>
<td>Overall symptoms</td>
<td>1.0 (0.5)</td>
<td>1.1 (0.5)</td>
</tr>
</tbody>
</table>

All values are mean (SD) adjusted for baseline using ANCOVA. Estimated mean differences and 95% CI’s are also presented.

\(^a\) Number of days on which each symptom was reported at moderate or severe during the final week of the diet; \(^b\) Average severity across 7 days; 0=absent, 1=mild, 2=moderate, 3=severe
5.3.2 **Stool output**

Stool frequency and consistency at end of trial in the ITT population are displayed in Table 5.5.

<table>
<thead>
<tr>
<th>Table 5.5 Stool frequency and consistency at end of trial in the ITT population (n=52)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low FODMAP diet (n=27)</td>
</tr>
<tr>
<td>Daily stool frequency</td>
</tr>
<tr>
<td>Bristol Stool Form Scale</td>
</tr>
<tr>
<td>Proportion normal stools a, %</td>
</tr>
<tr>
<td>Proportion loose stools a, %</td>
</tr>
<tr>
<td>Proportion hard stools a, %</td>
</tr>
</tbody>
</table>

All values are mean (SD) adjusted for baseline using ANCOVA. Estimated mean differences and 95% CI’s are also presented

a Normal (Bristol Stool Type 3, 4 or 5); loose (Bristol Stool Type 6 or 7); Hard (Bristol Stool Type 1 or 2)

There was significantly lower daily stool frequency at end of trial in the low FODMAP diet (1.7 SD 0.5) compared with the sham diet group (2.1 SD 0.5) (P=0.012), however there were no differences in stool consistency or the proportion of stools that were normal, loose or hard at end of trial. Similarly, significantly lower daily stool frequency was observed in the low FODMAP diet (1.8 SD 0.8) compared to the sham diet group (2.1 SD 1.1) (P=0.022) at end of trial in the PP analysis.

There was a significantly lower daily stool frequency at end of trial in the low FODMAP diet (1.7 SD 1.0) compared to the sham diet group (2.1 SD 1.1) (P=0.019) in patients with CD but not in UC (1.8 SD 0.6 vs. 2.0 SD 0.9, P=0.501). There was no difference in stool consistency between the diet groups in CD or UC at end of trial (data not shown).

5.4 **Clinical disease activity**

There was no difference in clinical disease activity between the low FODMAP diet and sham diet groups at baseline (Table 5.2) or at the end of trial for CD (mean HBI score 3.2 SD 1.9 vs. 3.4 SD 1.9, P=0.814) or UC (mean Partial Mayo Score 0.2 SD 0.4 vs. 0.2 SD 0.7, P=0.951). There were no differences in scores on the individual questions of the HBI or Partial Mayo score between the groups at baseline or end of trial (data not shown).

5.5 **Health-related quality of life and food-related quality of life**

There was a significantly higher end of trial IBDQ score (implying greater quality of life) in the low FODMAP diet group (81.9 SD 8.3) compared to the sham diet group (78.3 SD 7.5) (P=0.042) (Table 5.6). Specifically, there was a significantly higher score in the bowel II domain of the IBDQ in the low FODMAP diet group (76.5 SD 13.0) compared to the sham diet group (70.0 SD 9.5)
(P=0.031), however no other IBDQ domains (emotional, social, bowel I, systemic) were different between the diet groups (Table 5.6).

There was a higher total IBDQ score in the low FODMAP diet (83.0 SD 6.1) compared to the sham diet group (77.9 SD 6.1) (P=0.010) in the PP population, as well as a significantly higher score in the bowel I (87.3 SD 11.4 vs. 81.8 SD 10.9, P=0.029) and bowel II (79.4 SD 13.2 vs 70.3 SD 9.0, P=0.007) domains. There was no significant difference between the diet groups in total IBDQ score or sub-scores in CD and UC (data not shown).

It was hypothesised that an improvement in GI symptom severity may predict an improvement in HR-QOL. Univariate linear regression revealed that end of trial IBS-SSS score did indeed predict end of trial IBDQ score $F(1,50) = 56.1$ (P<0.001). The model accounted for 51% of the explained variability in end of trial IBDQ score. For each one-point decrease in the IBS-SSS, there was a predicted 0.08-point increase in IBDQ score.

Table 5.6 UK IBDQ total score and domain scores at end of trial in the ITT population (n=52)

<table>
<thead>
<tr>
<th></th>
<th>Low FODMAP diet group (n=27)</th>
<th>Sham diet group (n=25)</th>
<th>Mean difference (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total score</td>
<td>81.9 (8.3)</td>
<td>78.3 (7.5)</td>
<td>3.6 (0.1, 7.0)</td>
<td>0.042</td>
</tr>
<tr>
<td>Emotional</td>
<td>81.7 (9.4)</td>
<td>79.8 (9.0)</td>
<td>1.9 (-2.2, 2.2)</td>
<td>0.352</td>
</tr>
<tr>
<td>Bowel I</td>
<td>86.1 (12.0)</td>
<td>81.8 (10.5)</td>
<td>4.3 (-0.2, 8.9)</td>
<td>0.061</td>
</tr>
<tr>
<td>Social</td>
<td>93.8 (9.4)</td>
<td>90.6 (10.5)</td>
<td>3.2 (-1.3, 7.6)</td>
<td>0.157</td>
</tr>
<tr>
<td>Bowel II</td>
<td>76.5 (13.0)</td>
<td>70.0 (9.5)</td>
<td>6.5 (0.6, 12.4)</td>
<td>0.031</td>
</tr>
<tr>
<td>Systemic</td>
<td>62.3 (16.6)</td>
<td>58.0 (13.0)</td>
<td>4.3 (-2.0, 10.7)</td>
<td>0.176</td>
</tr>
</tbody>
</table>

Table values are mean (SD) adjusted for baseline using ANCOVA. Estimated mean differences and 95% CI’s are also presented.

At end of trial, there was no significant difference in FR-QOL score between the low FODMAP diet (77.3 SD 15.6) and sham diet (82.8, SD 18.5) (P=0.164). The results were similar in the PP population. Sub-group analyses showed no significant different between diet groups in CD or UC (data not shown).

5.6 IBD-control questionnaire

There was a significantly greater IBD-control score at end of trial in the low FODMAP diet (88.3 SD 22.3) compared to the sham diet (74.3 SD 25.0) (P=0.028). Sub-group analysis revealed that this was specific to patients with UC (94.2 SD 21.3 vs. 71.3 SD 25.6, P=0.022) and was not evident in CD (81.4 SD 24.0 vs. 79.1 SD 22.5, P=0.768). The results were similar in the PP population.

It was hypothesised that the IBS-SSS score at end of trial may be associated with the IBD-control score at follow-up, reflecting an improvement in GI symptoms despite no change in GI
inflammation. A linear regression showed that the IBS-SSS score was significantly associated with end of trial IBD-control score $F(1, 50) = 49.7, P<0.001$, accounting for 48.9% of the explained variability in IBD-control score.

5.7 Nutrient intake

Seven-day food records were obtained from all 52 participants at baseline and from 45 participants at end of trial (6 withdrawn from the trial, 1 failed to return food record). All dietary data were analysed ITT. Since one coder entered all 97 food diaries, it was not necessary to check inter-observer agreement. This section describes the effect of the low FODMAP and sham dietary advice on nutrient intakes.

Table 5.7 displays nutrient intakes at the end of trial (adjusted for baseline intakes). End of trial daily energy intake was significantly lower in the low FODMAP diet (1697 SD 514) than the sham diet group (1918 SD 624) ($P=0.002$). There was significantly lower protein and fat intake in the low FODMAP diet compared to the sham diet group. However, there was no difference between the low FODMAP diet and sham diet groups in the proportion of energy derived from carbohydrate (42% SD 6% vs. 41% SD 7%, $P=0.687$), protein (18% SD 4% vs. 18% SD 4%, $P=0.442$) or fat (36% SD 6% vs. 37% SD 6%, $P=0.442$). There was significantly lower sugar, calcium, phosphorous and iodine intake in the low FODMAP diet compared to the sham diet at end of trial.

To specifically investigate the effect of the low FODMAP diet on nutrient intake, baseline and end of trial nutrient intakes were compared in this group. The intake of energy (1640 SD 514 kcal/d vs. 1867 SD 593 kcal/d, $P=0.001$), protein (74 SD 18 g/d vs. SD 28 g/d, $P=0.024$), fat (66 SD 26g/d vs. 75 SD 27 g/d, $P=0.018$), carbohydrate (170 SD 60 g/d vs. 195 SD 69 g/d, $P=0.001$), calcium (664 SD 279 mg/d vs. 801 SD 308 mg/d, $P=0.006$), sodium (1469 SD 531 mg/d vs. 2095 SD 860 mg/d, $P<0.001$) and phosphorous (1131 SD 309 mg/d vs. 1277 SD 401 mg/d, $P=0.005$) significantly declined between baseline and end of trial.

The proportion of participants meeting national macronutrient, micronutrient and fibre recommendations was not different between the diet groups at end of trial (DoH, 1991), or between baseline and end of trial in the low FODMAP diet and sham diet groups (compared using a McNemar’s test) (data not shown).
Table 5.7 Seven-day average nutrient intakes in the low FODMAP and sham diet groups at end of trial in the ITT population (n=52)

<table>
<thead>
<tr>
<th>Metric</th>
<th>Low FODMAP diet</th>
<th>Sham diet</th>
<th>Mean difference (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>1697 (514)</td>
<td>1918 (624)</td>
<td>-220 (-357, -85)</td>
<td>0.002</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>74 (18)</td>
<td>83 (25)</td>
<td>-9 (-16, -2)</td>
<td>0.008</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>68 (26)</td>
<td>80 (36)</td>
<td>-11 (-22, -1)</td>
<td>0.035</td>
</tr>
<tr>
<td>Saturated fat (g)</td>
<td>24 (12)</td>
<td>27 (15)</td>
<td>-4 (-8, 1)</td>
<td>0.102</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>180 (60)</td>
<td>197 (72)</td>
<td>-17 (-34, 1)</td>
<td>0.058</td>
</tr>
<tr>
<td>Starch (g)</td>
<td>116 (36)</td>
<td>118 (50)</td>
<td>-1 (-15, 12)</td>
<td>0.841</td>
</tr>
<tr>
<td>Sugars (g)</td>
<td>63 (30)</td>
<td>76 (33)</td>
<td>-13 (-24, -2)</td>
<td>0.022</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>18 (6)</td>
<td>19 (8)</td>
<td>-1 (-4, 1)</td>
<td>0.249</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>692 (279)</td>
<td>911 (361)</td>
<td>-219 (-332, -106)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>11 (5)</td>
<td>12 (4)</td>
<td>-1 (-3, 1)</td>
<td>0.170</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>9 (6)</td>
<td>10 (4)</td>
<td>-1 (-4, 2)</td>
<td>0.470</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>1533 (531)</td>
<td>2196 (896)</td>
<td>-663 (-910, -415)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>2939 (1106)</td>
<td>3034 (937)</td>
<td>-95 (-526, 335)</td>
<td>0.658</td>
</tr>
<tr>
<td>Phosphorous (mg)</td>
<td>1140 (310)</td>
<td>1313 (402)</td>
<td>-173 (-276, -69)</td>
<td>0.002</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>290 (97)</td>
<td>297 (109)</td>
<td>-7 (-44, 30)</td>
<td>0.709</td>
</tr>
<tr>
<td>Iodine (μg)</td>
<td>124 (59)</td>
<td>176 (372)</td>
<td>-51 (-95, -8)</td>
<td>0.022</td>
</tr>
<tr>
<td>Selenium (μg)</td>
<td>59 (29)</td>
<td>57 (22)</td>
<td>1 (-11, 13)</td>
<td>0.823</td>
</tr>
<tr>
<td>Vitamin A (μg)</td>
<td>1358 (1005)</td>
<td>1328 (3599)</td>
<td>30 (-573, 632)</td>
<td>0.921</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>90 (54)</td>
<td>75 (48)</td>
<td>15 (-6, 36)</td>
<td>0.166</td>
</tr>
<tr>
<td>Vitamin D (μg)</td>
<td>6 (2)</td>
<td>6 (23)</td>
<td>0 (-1, 1)</td>
<td>0.818</td>
</tr>
<tr>
<td>Vitamin B1 (thiamin) (mg)</td>
<td>2 (1)</td>
<td>1 (0)</td>
<td>0 (-0, 0)</td>
<td>0.963</td>
</tr>
<tr>
<td>Vitamin B2 (riboflavin) (mg)</td>
<td>2 (1)</td>
<td>2 (1)</td>
<td>0 (-0, 0)</td>
<td>0.182</td>
</tr>
<tr>
<td>Vitamin B3 (niacin) (mg)</td>
<td>20 (6)</td>
<td>20 (7)</td>
<td>-1 (-4, 3)</td>
<td>0.672</td>
</tr>
<tr>
<td>Vitamin B6 (pyridoxine) (mg)</td>
<td>2 (1)</td>
<td>2 (1)</td>
<td>0 (-0, 0)</td>
<td>0.634</td>
</tr>
<tr>
<td>Vitamin B7 (biotin) (μg)</td>
<td>36 (18)</td>
<td>36 (15)</td>
<td>0 (-7, 7)</td>
<td>0.993</td>
</tr>
<tr>
<td>Vitamin B9 (folate) (μg)</td>
<td>229 (85)</td>
<td>257 (108)</td>
<td>-28 (-62, 7)</td>
<td>0.110</td>
</tr>
<tr>
<td>Vitamin B12 (cobalamin) (μg)</td>
<td>6 (7)</td>
<td>6 (2)</td>
<td>0 (-2, 3)</td>
<td>0.782</td>
</tr>
</tbody>
</table>

All values are mean (SD) adjusted for baseline using ANCOVA

5.7.1 Body weight

There was a significantly lower body weight in the low FODMAP (71 SD 14 kg) compared to the sham diet group at end of trial (73 SD 12 kg) (P <0.001). In the sham diet group, end of trial body weight (74.8 SD 12.1 kg) was significantly higher than baseline (74 SD 12 kg) (P=0.015), while in the low FODMAP diet group end of trial body weight (70.0 SD 14.4 kg) was significantly lower than baseline (70.7 SD 15.0 kg) (P=0.015).
5.8 FODMAP intake

Fermentable carbohydrate intakes at end of trial were analysed ITT and are presented in Table 5.8. Total FODMAP intake was calculated as the sum of fructans, GOS, lactose, fructose in excess of glucose and polyols (sorbitol and mannitol). Total and individual FODMAP intakes were significantly lower in the low FODMAP diet than in the sham diet group at end of trial.

Table 5.8 Total and individual fermentable carbohydrate intakes at end of trial in the ITT population (n=52)

<table>
<thead>
<tr>
<th>Fermentable carbohydrate</th>
<th>Low FODMAP diet (n=27)</th>
<th>Sham diet (n=25)</th>
<th>Mean difference (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>8.1 (7.5)</td>
<td>17.9 (10.3)</td>
<td>-9.8 (-13.2, -6.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Change in total FODMAPs&lt;sup&gt;a&lt;/sup&gt; from baseline&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-7.9 (8.6)</td>
<td>2.3 (4.8)</td>
<td>-9.8 (-13.2, -6.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fructans</td>
<td>1.3 (0.9)</td>
<td>2.9 (1.3)</td>
<td>-1.6 (-2.2, -1.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GOS</td>
<td>0.4 (0.3)</td>
<td>0.9 (0.6)</td>
<td>-0.4 (-0.7, -0.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lactose</td>
<td>5.8 (7.1)</td>
<td>10.7 (9.9)</td>
<td>-5.3 (-8.3, -2.3)</td>
<td>0.001</td>
</tr>
<tr>
<td>Excess fructose</td>
<td>0.5 (0.5)</td>
<td>1.3 (1.3)</td>
<td>-0.9 (-1.4, -0.4)</td>
<td>0.001</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>0.2 (0.3)</td>
<td>0.6 (0.8)</td>
<td>-0.5 (0.2, 0.8)</td>
<td>0.001</td>
</tr>
<tr>
<td>Mannitol</td>
<td>0.1 (0.2)</td>
<td>0.3 (0.3)</td>
<td>-0.2 (-0.3, -0.1)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Values are mean (SD) grams per day adjusted for baseline using ANCOVA
<sup>a</sup> Change in total FODMAP intake between baseline and end of trial

Total and individual FODMAPs were significantly lower at end of trial compared to baseline in the low FODMAP diet group (Figure 5.4) while in the sham diet group there was no difference between baseline and end of trial (data not shown).

![Figure 5.4 Fermentable carbohydrate intakes at baseline and end of trial in the low FODMAP diet group. Baseline and end of trial intakes were compared using the Wilcoxon Signed Rank test. All P<0.05 for baseline vs. end of trial. Fructose in excess of glucose is presented](image-url)
5.9 Acceptability

For most of the diet acceptability questions, significantly more patients in the low FODMAP diet group answered the question negatively compared to the sham diet group, except for meal preparation and understanding the written material provided during the trial (Table 5.9). Most of the participants in both groups reported that the benefits of being involved in the research outweighed the burden of taking part (84% low FODMAP diet vs. 64% in the sham diet group, P=0.180).

Table 5.9 Diet acceptability responses at end of trial in patients completing the trial (n=46)

<table>
<thead>
<tr>
<th></th>
<th>Low FODMAP diet (n=24)</th>
<th>Sham diet (n=22)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meal preparation was more difficult</td>
<td>18 (72)</td>
<td>10 (36)</td>
<td>0.081</td>
</tr>
<tr>
<td>Longer time spent shopping for food</td>
<td>20 (80)</td>
<td>8 (36)</td>
<td>0.003</td>
</tr>
<tr>
<td>Longer time spent preparing and cooking meals</td>
<td>14 (56)</td>
<td>5 (23)</td>
<td>0.036</td>
</tr>
<tr>
<td>Finding suitable foods when eating out was more difficult</td>
<td>23 (92)</td>
<td>13 (59)</td>
<td>0.014</td>
</tr>
<tr>
<td>The flavour of meals and snacks was less appealing</td>
<td>15 (60)</td>
<td>2 (9)</td>
<td>0.001</td>
</tr>
<tr>
<td>More money spent on shopping and eating out</td>
<td>20 (80)</td>
<td>1 (5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>The written information was difficult to understand</td>
<td>0 (0)</td>
<td>1 (5)</td>
<td>0.468</td>
</tr>
<tr>
<td>The diet was less convenient</td>
<td>20 (80)</td>
<td>15 (32)</td>
<td>0.001</td>
</tr>
<tr>
<td>The diet was more difficult</td>
<td>22 (88)</td>
<td>8 (36)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lots of dietary changes were made</td>
<td>22 (88)</td>
<td>11 (50)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Values are number of participants (% of group) with a negative response
Groups were compared using a Chi-squared test

5.10 Blinding

At end of trial, participants were asked whether they were able to identify which diet group they had been allocated to (‘intervention diet’, ‘placebo diet’ or ‘not sure’). Of all the patients completing the trial, 34/46 (74%) correctly identified their diet allocation. In the low FODMAP diet group, 14/24 (58%) correctly identified diet allocation compared to 20/22 (91%) in the sham diet group (P=0.034). Two patients in the low FODMAP diet group (8%) and one patient in the sham diet group (5%) were ‘not sure’ of their diet allocation (P>0.05).

To determine the level of agreement between participants’ actual diet allocation and perceived diet allocation, Cohen’s κ was computed between diet allocation and perceived diet allocation. For the purpose of this analysis, participants who answered ‘not sure’ (3/52, 6%) of their diet allocation were classified as guessing incorrectly. There was moderate agreement between the actual and perceived diet allocation, $\kappa = 0.485$ (95% CI 0.25, 0.72), P < 0.001 (Landis and Koch, 1977).
Chapter 5: RCT clinical and dietary intake results

5.11 Discussion and conclusion

5.11.1 Gastrointestinal symptoms

The null hypothesis of this trial was that there is no difference in the change in symptom severity score (IBS-SSS) at four weeks following low FODMAP dietary advice compared to placebo sham dietary advice in patients with inactive IBD with FGS. This hypothesis must be accepted since there was no significant difference between the groups. However, the difference between groups approached statistical significance (P=0.075) with a greater reduction in score in the low FODMAP diet group. Additionally, there were numerous findings suggestive of an improvement in GI symptoms following the low FODMAP diet, including more patients with adequate relief of GI symptoms, lower bloating and flatulence severity, and greater patient-perceived control of IBD following the low FODMAP diet compared to the sham diet.

An improvement in global GI symptoms (adequate relief) and specifically bloating and flatulence following the low FODMAP diet is supported mechanistically. Fructan challenges increase colonic gas volume and fructose and polyols increase small intestinal water content in healthy individuals and patients with IBS, with resulting GI symptoms experienced by patients with IBS (Major et al., 2017, Murray et al., 2004, Marciani et al., 2010). Although colonic gas generation following α-GOS ingestion has not been directly studied using, for example, MRI, α-GOS are capable of increasing flatulence in healthy volunteers (Steggerda et al., 1966, Suarez et al., 1999). As explained in Chapter 1 of this thesis, colonic gas generation following FODMAP consumption is hypothesised to occur through colonic bacterial fermentation of FODMAPs, which likely varies considerably among different FODMAPs and even different degrees of polymerisation of fructans and GOS (Hernot et al., 2009).

It is therefore unsurprising that pure fructan and fructose challenges increased global GI symptoms and fructans increased bloating and flatulence following a low FODMAP diet in patients with IBS (Shepherd et al., 2008). Similarly, the re-challenge trial described in Chapter 3 of this thesis demonstrated for the first time that fructan challenge increased abdominal pain, bloating and flatulence in patients with inactive IBD following FODMAP restriction, indicating similar effects of FODMAPs in patients with FGS on the background of inactive IBD (Cox et al., 2017). Furthermore, numerous studies have observed improved GI symptoms including abdominal bloating and flatulence following the low FODMAP diet in IBS (Staudacher et al., 2012, Staudacher et al., 2017b, Halmos et al., 2014b, Bohn et al., 2015, Eswaran et al., 2016) and uncontrolled studies have suggested a similar response in IBD (Gearry et al., 2009b, Prince et al., 2016). Indeed, abdominal bloating and flatulence demonstrated the greatest improvement following low FODMAP dietary advice in the latter prospective study. Furthermore, a large proportion (54%) of patients in the current trial fulfilled the criteria for functional bloating at
enrolment and an improvement in bloating was therefore possible and may have resulted in adequate relief at end of trial.

It is promising that more patients reported adequate relief following the low FODMAP diet compared to the sham diet. This outcome permits a global assessment of GI symptoms that can be extremely heterogeneous in IBS and IBD and, since it reflects the overall tolerability of GI symptoms, is arguably of great clinical significance. Adequate relief has therefore been widely utilised in IBS trials (Mangel et al., 1998) and continues to be recommended by the Rome committee for the assessment of GI symptoms in FGID because it demonstrates excellent construct validity (Irvine et al., 2016, Spiegel et al., 2009).

Adequate relief permits comparison of trial results since it overcomes the heterogeneity in GI symptom responses to treatments. This has has been demonstrated in trials of linaclotide for constipation-predominant constipation (Camilleri et al., 2015). The previous placebo-controlled trial of the low FODMAP diet in IBS revealed similar proportions of patients reporting adequate relief in the low FODMAP diet group (57% vs. 52% in the current trial), but a greater proportion of patients in the sham diet group reported adequate relief in the previous trial (38% vs. 16% in the current trial) (Staudacher et al., 2017b). This may reflect differences in GI symptom perception and validity of the adequate relief outcomes between IBS and IBD. Indeed, adequate relief has not been validated in patients with IBD, although it has been recently used to demonstrate an improvement in GI symptoms following low FODMAP dietary advice and a worsening of GI symptoms following fructan challenge in patients with inactive IBD (Prince et al., 2016, Cox et al., 2017).

Meanwhile, the difference in the change in IBS-SSS between baseline and end of trial failed to reach significance between the diet groups. This is discordant with recent trials in both IBD (Pedersen et al., 2017) and IBS (Staudacher et al., 2017b) and may be explained in several ways. The recent trial in patients with FGS in IBD (Pedersen et al., 2017) was unblinded and thus achieved a greater difference between the diet groups as a result of a placebo response in the low FODMAP diet group that was not present in the control group. Moreover, a full explanation of the mechanisms and theory of the low FODMAP diet was provided to patients in the intervention group, which may have inflated the placebo response. These differences in trial design could explain the more modest difference observed between the low FODMAP diet and sham diet in the current study, in which sham dietary advice was provided to patients in the control group and in which patients simply received a list of foods permitted, without an explanation of the mechanisms.
There was no difference in abdominal pain incidence or severity between the diet groups in this trial. Luminal distension associated with bacterial gas production would be expected to result in abdominal pain as well as bloating and flatulence. Indeed, previous studies of the low FODMAP diet in IBS and IBD observed an improvement in abdominal pain (Halmos et al., 2014b, Staudacher et al., 2017b, McIntosh et al., 2016, Bohn et al., 2015, Eswaran et al., 2016, Pedersen et al., 2017) and fructan challenge increased abdominal pain (in addition to bloating and flatulence) in patients with IBD in the second chapter of this thesis. A lack of improvement in abdominal pain in this trial may reflect alternative mechanisms of pain induction not related to luminal distension, such as low-grade inflammation (Vivinus-Nébot et al., 2014), nerve receptor sensitisation (Lapointe et al., 2015) and on-going undetected small bowel complications such as strictures in CD. Secondly, and likely most importantly, 38% of participants met the criteria for IBS at baseline, while the remainder met the criteria for functional bloating (54%) and functional diarrhoea (8%). Up to 62% of participants may therefore have had no abdominal pain at baseline, which of course would preclude an improvement in this symptom. Finally, as previously discussed, prior experience of severe recurrent abdominal pain during IBD relapses may result in differing perceptions of abdominal pain compared to patients with IBS and may influence abdominal pain ratings in patients with a background of IBD.

While the most recently published trial of the low FODMAP diet in IBS used the same sham dietary advice as the current study (Staudacher et al., 2017b), the discordant IBS-SSS findings compared to the current trial could reflect differences in IBS and IBD pathophysiology or differing ability of the IBS-SSS to accurately represent symptoms in IBD, a population in which this questionnaire has not been validated. Interestingly, the IBS trial failed to show a significant difference in adequate relief, in contrast to the current trial. This further supports differing validity of GI symptom outcome measures in IBS and IBD. Prior experience of GI symptoms during IBD relapses may shape the likelihood of reporting adequate relief, although this is yet to be formally evaluated (Prince et al., 2016, Cox et al., 2017).

Although there was no significant difference between the diet groups in the proportion of patients achieving the MCID (50-point reduction) in the IBS-SSS, more patients in the low FODMAP diet group achieved at least a 50% reduction in IBS-SSS score during the trial. This could reflect a placebo response in the sham diet group narrowing the difference between the groups for the MCID; i.e. lots of patients achieved a small reduction in score in both groups (50 points) but few patients achieved a drastic reduction (≥50%). The proportion of patients achieving the MCID in the sham diet group in this trial (36%) is similar to the previous trial in IBS (42%) (Staudacher et al., 2017b). However, the proportion achieving the MCID in the low FODMAP diet group in this trial (56%) was lower than in the previous trial in IBS (73%) (Staudacher et al.,
These differences could relate to differences in GI symptom responses in IBS and IBD and the lack of blinding in the previous IBD trial.

There was also discrepancy between the same GI symptoms measured using the IBS-SSS and the GSRS within this trial. Bloating was significantly lower following the low FODMAP diet when measured using the IBS-SSS but not the GSRS. There was however lower severity of flatulence on the GSRS in the low FODMAP diet group. While neither of these outcome measures have been validated in patients with IBD specifically, they have been used to demonstrate GI symptom improvements following low FODMAP dietary advice and to evaluate the prevalence of GI symptoms in patients with inactive IBD (Prince et al., 2016, Simrén et al., 2002). The observed differences in GI symptoms measured using these outcome measures could reflect questionable validity in detecting symptom changes in IBD, different measurement scales, or a difficulty in separating bloating and flatulence. In support of this, cognitive interviews have demonstrated that patients with IBS struggle to differentiate pain and discomfort, and that bloating involves both the sensation and the visual appearance (Spiegel et al., 2010).

5.11.2 Gastrointestinal symptoms according to diagnosis, disease location and baseline GI inflammation

Based upon the adequate relief outcome, 48% of patients did not respond to the low FODMAP diet in this trial. The ability to predict which patients are most likely to respond to low FODMAP dietary advice is attractive in clinical practice since it would reduce the number of patients unnecessarily attempting this diet, which can be challenging to execute.

Sub-group analyses revealed a significantly greater reduction in IBS-SSS score during the trial and a significantly lower end of trial IBS-SSS score in the low FODMAP diet compared to the sham diet group in patients with UC but not CD. Although this trial was not powered to detect differential effects of the low FODMAP diet in patients with CD and UC, the lack of effect in patients with CD is interesting and there are several possible explanations for this. Firstly, the pathophysiology of CD and UC differ substantially and it is therefore unsurprising that both drug treatments (Cholapranee et al., 2017) and dietary treatments (Derwa et al., 2017, Narula et al., 2018) have demonstrated differing efficacy in CD and UC. Secondly, the low FODMAP diet is hypothesised to improve GI symptoms through reducing luminal distension associated with gas generated through colonic bacterial fermentation (Staudacher and Whelan, 2017). Animal models have shown that alterations in neurophysiology and GI motility can persist following resolution of inflammation (Lapointe et al., 2015, Krauter et al., 2007) and the reduced distension in the colon following the low FODMAP diet may therefore have a greater potential to benefit patients with UC. That said, a large proportion of patients with CD in the low FODMAP diet group in this trial had some degree of colonic involvement (71% with either ileocolonic or
colonic CD). In line with this hypothesis, sensitivity analysis revealed a significantly greater reduction in IBS-SSS score during the trial upon exclusion of patients with isolated ileal CD (leaving only patients with either UC and colonic or ileocolonic CD).

Thirdly, patients were defined as in remission and therefore suitable for enrolment in the trial based upon faecal calprotectin concentration. There are studies supporting and opposing the ability of faecal calprotectin to detect isolated small intestinal inflammation (Sipponen et al., 2012, Arai et al., 2017) and the small intestine is less accessible and more challenging to assess through endoscopy. Therefore some patients with CD may have had on-going small intestinal inflammation not detected through faecal calprotectin, which, as discussed above, could have abrogated GI symptom responses to the diet in the patients with CD. Finally, fibrotic strictures can result from prior transmural GI inflammation in CD and can cause GI symptoms even in the absence of active inflammation (Van Assche et al., 2010). A recent endoscopy or MRI was not required prior to trial enrolment and some patients with CD may have had strictures that could confound GI symptom responses, although patients with known strictures were excluded.

Although there was no difference in the change in the IBS-SSS score between the low FODMAP and sham diet groups at end of trial, a sensitivity analysis including only patients with IBD with colonic involvement (i.e. UC, colonic CD, ileocolonic CD) resulted in a significantly larger reduction in IBS-SSS in the low FODMAP diet group. This suggests the presence or absence of colonic disease may influence GI symptom responses to the low FODMAP diet. A possible explanation may be lasting effects of prior colonic inflammation that may influence the likelihood of GI symptoms following bacterial fermentation and colonic distension. It could be that patients with colonic disease are more likely to respond to dietary fermentable carbohydrate restriction and a reduction in colonic distension. For the same reason, these patients may habitually avoid or reduce the intake of high FODMAP foods, supported by lower GOS and polyol intakes in inactive IBD with FGS in the case-control study in Chapter 2. This may also explain the observed association between a lower fructan intake with the presence of colonic disease observed in the case-control study, but this requires further investigation in larger studies.

Change in IBS-SSS score between baseline and end of trial was positively correlated with baseline faecal calprotectin. This suggests that a lower degree of intestinal inflammation was associated with a greater reduction in IBS-SSS score (and thus, an improvement in GI symptoms) during the trial. This finding is congruent with the aforementioned IBD trial in which there was no difference in change in IBS-SSS score between low FODMAP diet and habitual diet in patients with elevated faecal calprotectin at baseline (>100 μg/g in UC and >200 μg/g in CD) (Pedersen et al., 2017), although acknowledging that only 23% of participants had elevated faecal calprotectin at baseline (Pedersen et al., 2017).
baseline. Unfortunately, the small numbers of participants with faecal calprotectin above 50 μg/g and 100 μg/g in the current trial prevented meaningful sub-group analyses of symptom responses according to baseline faecal calprotectin. The aetiology of GI symptoms in the presence of even subtle active intestinal inflammation may differ from truly ‘functional’ symptoms; for example, abdominal pain during inflammation is likely the result of primary afferent neuron sensitisation by inflammatory cytokines and mediators (Farrell et al., 2014) and whether patients with mildly active IBD could benefit from the low FODMAP diet requires further investigation.

5.11.3 Stool output

There was a lower stool frequency following the low FODMAP diet compared to the sham diet in this trial, however there was no difference in stool consistency. The difference in stool frequency following the low FODMAP diet was somewhat expected on the basis that certain fermentable carbohydrates, primarily fructose and polyols, increase small bowel water (Major et al., 2017, Murray et al., 2014, Marciani et al., 2010). Furthermore, certain fermentable carbohydrates may influence GI motility. For example, a 30 g fructose-sorbitol mixture increased GI motility in healthy volunteers (Madsen et al., 2006). Finally, inulin and GOS are types of dietary fibre and have been shown to influence stool output (Brownlee, 2011, Holscher et al., 2014, Micka et al., 2017, Silk et al., 2009), albeit in healthy volunteers with and without constipation and patients with IBS in whom GI physiology may differ to patients with IBD. The low FODMAP diet has also been shown to alter stool frequency (Halmos et al., 2014b) and consistency (Staudacher et al., 2012, Staudacher et al., 2017b) in some trials in patients with IBS.

The lack of effect on stool consistency following the low FODMAP diet in this trial is discordant with the re-challenge trial described in Chapter 3 of this thesis that demonstrated softer stool consistency following fructan and GOS challenges. There are several possible explanations for this. Firstly, the GI tract may be able to accommodate additional luminal water in excess of that likely to be introduced through osmotically active carbohydrates (Kamath et al., 1990). Furthermore, short and long-chain fructans have differential effects on whole gut transit time in healthy volunteers, indicating that the degree of polymerisation of the fermentable carbohydrate challenges in the re-challenge trial may produce discrepancies compared to a low FODMAP diet trial (Rumessen and Gudmand-Hoyer, 1998). Similarly, fermentable carbohydrate challenge or supplementation does not mimic the effect of broad restriction of fermentable carbohydrates within whole foods.

A further consideration is the small proportion of abnormal stools at baseline (35% of stools were classified as ‘loose’ or ‘hard’ during the screening week) in the current trial and the fact that 16% of the sham diet group fulfilled the criteria for functional diarrhoea compared to 0% in
the low FODMAP diet group. This group of patients were experiencing regular persistent diarrhoea and were confined to the sham diet group, which may have limited the ability to detect an effect of the low FODMAP diet on stool consistency.

### 5.11.4 Clinical disease activity

There was no significant difference in clinical disease activity between the diets at the end of trial. Given that much of the HBI is based upon GI symptoms (loose stools and abdominal pain) and general well-being, it is unsurprising that the mean baseline scores in this trial exceeded the typical threshold for disease in remission (a total score of ≤4 is often cited as an appropriate cut-off for inactive CD since this corresponds to a CDAI ≤150 points (Vermeire et al., 2010)). This may reflect the fact that patients were recruited to this trial based upon the presence of FGS. In contrast, mean baseline Partial Mayo scores in both diet groups were below the suggested ≤1 point cut-off for remission (Peyrin-Biroulet et al., 2015). This is likely because two of the three questions in this measure relate to Physician Global Assessment and rectal bleeding, both of which should have low scores in the context of patients in remission. Although sub-group analyses revealed a greater reduction in GI symptom severity in patients with UC during the trial, the end of trial Partial Mayo score is unlikely to reflect this since this score is less responsive to GI symptom report than the HBI. In contrast, the Simple Clinical Colitis Activity Index (SCCAI) assesses general well-being, stool frequency (daytime and nocturnal) and faecal urgency and had it been used scores could be increased by the presence of FGS.

### 5.11.5 Health-related quality of life and food-related quality of life

There was a significantly higher IBDQ score in the low FODMAP diet group at end of trial, suggesting greater HR-QOL in this group. As expected, end of trial symptom severity was significantly associated with IBDQ score. These findings mirror those of a recent trial demonstrating a significant improvement in HR-QOL following the low FODMAP diet in IBD (Pedersen et al., 2017). Furthermore, HR-QOL score following exclusive enteral nutrition has been shown to correlate negatively with the CDAI score in active CD (Guo et al., 2013). Improvements in HR-QOL were also observed following medical and surgical treatments in a large cohort of newly diagnosed European patients with IBD, suggesting that management of inflammation and GI symptoms improves HR-QOL (Burisch et al., 2014b).

The Bowel II domain of the IBDQ scored significantly higher in the low FODMAP diet group at end of trial. This is unsurprising given that this domain encompasses the effect of GI symptoms (including abdominal pain, bloating, flatulence and nausea), which other GI symptoms measures showed to be reduced following the diet, on HR-QOL. The lack of improvement in the emotional and social function domains is interesting given that several previous studies have noted poorer HR-QOL in these domains in patients with FGS in IBD compared to those without FGS (Gracie et
This may be explained by different coping styles among different patients; a systematic review demonstrated varying coping styles among patients with IBD, with some displaying a problem-focused and others an emotion-focused coping style, the latter of which is generally associated with poorer outcomes (McCombie et al., 2013). The lack of effect of the low FODMAP diet on emotional and social domains of the IBDQ may relate to adaptive coping mechanisms in some patients with IBD (McCombie et al., 2016).

There are numerous IBD-specific HR-QOL instruments, including the IBDQ (Guyatt et al., 1989), the short-IBDQ (Irvine et al., 1996) and the Rating Form of IBD Patient Concerns (Drossman et al., 1991). A systematic review revealed that the IBDQ is the most widely used HR-QOL tool in the literature and performs well in terms of measurement properties and methodological quality, although certain other HR-QOL instruments were considered superior in terms of construct validity and responsiveness (Chen et al., 2017). Although the FGS experienced by patients enrolled in this trial are ‘IBS-like’, previous studies have generally utilised IBD-specific measures (rather than IBS-specific measures) in patients with IBD and FGS since these reflect domains of HR-QOL relevant to IBD (Pedersen et al., 2017, Abdalla et al., 2017, Bryant et al., 2011).

Given that dietary restrictions were required during the dietary interventions in this trial, it was important to investigate whether FR-QOL was affected. (Hughes et al., 2016). Interestingly, there was no significant difference in end of trial FR-QOL score or change in score at baseline and end of trial between the groups, despite dietary restrictions. This indicates that the low FODMAP diet and sham diet do not worsen FR-QOL in patients with IBD in whom this may already be lower than healthy individuals. This may be explained by an increase in perceived control over diet and GI symptoms during the trial that counterbalanced the negative effects of dietary restrictions on FR-QOL.

**5.11.6 Patient-reported outcome (IBD-control questionnaire)**

There was a significantly greater patient-perceived control of IBD in the low FODMAP diet group at end of trial, particularly in those with UC, where a reduced symptom severity was also observed. The IBD-control questionnaire was developed using patient reports of domains important to perceived control of IBD and therefore likely captures the aspects of IBD most important to them.

The improved patient-perceived control of IBD occurred despite no observed difference in clinical or biochemical disease activity between the groups, suggesting that this was a result of reduced FGS following the low FODMAP diet in UC. This was supported by a positive correlation between end of trial IBD control and IBS-SSS score.
This finding raises the inevitable issue that patients with IBD are unable to differentiate FGS from symptoms of active IBD, simply regarding these as symptoms of ‘IBD’. This reiterates both the clinical dilemma of differentiating FGS from active IBD and a potential limitation of using PROMs in patients with IBD and FGS. If clinical decisions were based entirely on PROMs, patients with FGS and no GI inflammation could receive inappropriate escalation of anti-inflammatory medication. Furthermore, a prospective study revealed that patients with more symptoms attributable to IBD had a higher self-reported IBD disability and this was associated with greater direct healthcare costs (van der Have et al., 2015).

5.11.7 Nutrient and FODMAP intake

Several previous studies have demonstrated that the low FODMAP diet influences nutrient intakes in patients with IBS, however to date this had not been replicated in IBD. Total energy intake was significantly lower following the low FODMAP diet in this trial, with no change in the proportion of energy derived from each macronutrient. Additionally, calcium, phosphorous and iodine intakes were lower following the low FODMAP diet compared to the sham diet. Nonetheless, the proportion of patients meeting recommendations or requirements for macronutrients, micronutrients and fibre was not different between the diet groups.

Reduced energy intake has previously been observed in patients with IBS in trials comparing the low FODMAP diet to standard IBS advice (Bohn et al., 2015, Eswaran et al., 2016, Zahedi et al., 2017). Other studies revealed no difference in energy intake following the low FODMAP diet compared to a habitual diet (Staudacher et al., 2012) and sham dietary advice (Staudacher et al., 2017b). Reduced energy intake likely relates to numerous dietary restrictions required during the low FODMAP diet, which in IBD may be on the background of existing dietary restrictions (Prince et al., 2011, Holt et al., 2017, Limdi et al., 2016). Patients with IBD are at increased risk of malnutrition (Lomer, 2011) and further compromising energy and nutrient intake may exacerbate existing malnutrition. Another important consideration is that human and murine models have suggested an important role of energy intake on the GI microbiota (Cotillard et al., 2013, Griffin et al., 2017). It is therefore possible that changes in energy intake in the low FODMAP diet group could have a confounding effect on the microbiota alterations that were observed (Chapter 6).

Reduced calcium intake has previously been observed following low FODMAP dietary advice in IBS (Staudacher et al., 2012), the likely explanation for which being the restriction of calcium-rich, lactose-containing dairy products. Although lactose-free cow’s milk and calcium-fortified milk alternatives (such as soya, almond and rice milk) are encouraged during the low FODMAP diet, clinical experience indicates that not all patients consume these products. This may relate to cost, convenience and taste, and patients may choose instead to consume foods that do not
require the addition of dairy products. For example, wheat-free toast or eggs may be chosen instead of cereal and milk for breakfast. Patients are also encouraged to consume non-dairy calcium-rich foods such as fish with bones and green, leafy vegetables, but these are unlikely to be consumed in sufficient quantities in a normal diet to replace the calcium derived from dairy foods. The full nutritional composition of some lactose-free specialty products is not available in the Composition of Foods database used in this trial, and this could reduce observed calcium intakes rather than actual intakes. Furthermore, the calcium intake in the sham diet group was higher than in previous studies of IBD (Vernia et al., 2014, Lim et al., 2014, Vidarsdottir et al., 2016), and this likely increased the difference in intake between the diet groups.

Nonetheless, the reduced calcium intake during the trial in the low FODMAP diet group may be detrimental since patients with IBD are at greater risk of bone fractures than the general population (Targownik et al., 2013, van Staa et al., 2003). These findings again suggest that vigilant dietary counselling and monitoring is vital and reintroducing high FODMAP foods after a maximum of 4 weeks is essential to correct reduced energy and calcium intakes (Whelan et al., 2018).

The aim of this trial was to reduce dietary fermentable carbohydrate intake in the low FODMAP diet group, while maintaining FODMAP intake in the sham diet group. The significantly lower FODMAP intake in the low FODMAP diet group compared to the sham diet group at end of trial and the reduced FODMAP intake between baseline and end of trial in the low FODMAP diet group (but not the sham diet group) indicates that this aim was achieved. Total daily FODMAP intake was 9.2 g lower in the low FODMAP diet group than the sham diet group at end of trial.

Several previous trials of the low FODMAP diet in IBS have estimated FODMAP intakes before and after the diet. The 9.2 g/d difference in FODMAP intake between the diet groups in the current trial is comparable to previous trials of low FODMAP dietary advice trials in the UK, which reflects that the dietary advice was comparable and analysis was performed using the same FODMAP database (Staudacher et al., 2017b, Staudacher et al., 2012). Interestingly, total baseline FODMAP intake (15.9 g/d) was similar to that of the control groups in the previous trials of the low FODMAP diet in IBS (17.4-17.7 g/d), suggesting that patients with IBD and FGS may have similar dietary FODMAP intake to patients with IBS. Providing participants with all foods during a trial facilitates much lower FODMAP intakes, as was demonstrated in an Australian trial in which total daily FODMAP intake was 3.05 g on the low FODMAP diet when all meals were provided (compared to 8.1 g/day in the current trial) (Halmos et al., 2014b). Some studies do not report on all fermentable carbohydrates (Eswaran et al., 2016, Zahedi et al., 2017) or do not assess dietary intake at all (McIntosh et al., 2016), which reduces the certainty that any observed symptom responses relate to a reduction in FODMAP intake specifically. Others use different
FODMAP composition databases to assess FODMAP intakes, limiting comparability between studies (Bohn et al., 2015).

5.11.8 Acceptability

Diet acceptability is a predictor of adherence and thus, influences the success of the diet. In a retrospective study of the low FODMAP diet in clinical practice in patients with IBD, GI symptom improvement was associated with adherence to the diet, highlighting the need to assess acceptability as a potential influencer of adherence (Gearry et al., 2009a). The low FODMAP diet appeared to be less acceptable to patients than the sham diet. In addition, more patients in the low FODMAP diet group reported making extensive dietary changes compared to the sham diet group, which may explain the poorer diet acceptability observed in the low FODMAP diet group. Nonetheless, the majority of patients in the low FODMAP diet group (84%) reported that the benefit of taking part in the research outweighed the burden, suggesting that symptom improvements and the positive impact of participating in research were sufficient to balance the difficulties of the diet.

Acceptability of a dietary intervention is determined by the patient's judgement of the advantages and disadvantages of the intervention, in terms of perceived benefits, cost, palatability and convenience (Berkow et al., 2010). Acceptability to vegan, vegetarian, Mediterranean, and a web-based gluten-free diet have been measured using a variety of tools (Moore et al., 2015, Lara et al., 2015, Sainsbury et al., 2015) but there is no validated tool to measure the acceptability of the low FODMAP diet specifically and surprisingly few studies have assessed the acceptability of the low FODMAP diet.

In clinical practice, more intensive dietary counselling on the low FODMAP diet is provided to patients requiring it (Whelan et al., 2018). Acceptability of the low FODMAP diet may be poorer in the research setting, particularly in a blinded trial, where a consistent period of time is spent on dietary counselling for each participant. Furthermore, in the interest of blinding, mechanisms of the diet were not explained to participants in this trial, and this may have reduced acceptability since the perceived benefit of the diet is lessened. The differences in diet acceptability between the low FODMAP diet and sham diet may relate to some of the permitted foods being staple foods in the UK (such as wheat and dairy), that were permitted to ensure no impact upon FODMAP or nutrient intakes. Unfortunately, this may have resulted in fewer dietary changes in the sham diet group, as evidenced by only 50% reporting extensive dietary changes (compared to 88% in the low FODMAP diet group). This may suggest that the sham diet may need to be adapted for future studies to match the low FODMAP diet in terms of difficulty and dietary changes.
5.11.9 Blinding
The proportion of participants in the low FODMAP diet group who were able to correctly identify their diet allocation (58%) is similar to what would be expected by chance, given that there are two possible diet allocations (50%). In contrast, the proportion of participants in the sham diet group able to correctly identify diet allocation was greater than would be expected by chance (91%). Many patients with IBD consider diet an important factor in terms of GI symptom induction and risk of relapse (Holt et al., 2017, Limdi et al., 2016, Prince et al., 2011). Questionnaire surveys have revealed that 27% of patients believe a dairy free diet can improve symptoms of active IBD, 73% had received dietary advice and 56% of patients modified their diet after diagnosis (Limdi et al., 2016, Zallot et al., 2013). This suggests that patients with IBD may have an enhanced awareness of the role of diet in IBD and GI symptom management and there may be aspects of the sham diet that triggered suspicion of diet allocation. For example, dairy products are a common perceived symptom trigger, yet were permitted on the sham diet. A more effective sham diet, in terms of blinding, would perhaps exclude dairy products and wheat. However, dairy is the source of the majority of lactose in the UK diet, and wheat is the greatest contributor to fructan intakes. Therefore, by restricting dairy and wheat, the diet would likely reduce FODMAP intakes, and would thus no longer be an effective placebo diet. A prospective study of patients' perceptions of the sham diet would be valuable to establish predictors for correctly identifying diet allocation, including whether non-response of symptoms is involved in assuming the sham diet is the placebo diet, and also to allow modification of the sham diet to enhance blinding.

5.11.10 Significance of results
The low FODMAP diet has the potential to provide benefit to many patients with IBD, given the success of the diet in IBS and the large proportion of patients with IBD in remission continuing to experience functional GI symptoms (Staudacher and Whelan, 2017, Halpin and Ford, 2012). This was the first ever placebo-controlled trial of the low FODMAP diet in IBD and demonstrates that the diet improves GI symptoms compared to a placebo diet in patients with inactive IBD, with 52% of patients reporting adequate relief. Although there was no difference between groups in the proportion reaching the MCID in the IBS-SSS, 56% of patients in the low FODMAP diet group reached the MCID, suggesting that the diet leads to clinically important improvements in GI symptoms in more than half of patients with IBD in remission.

Abdominal bloating and flatulence severity were significantly lower following the low FODMAP diet, which is clinically relevant given that 54% of participants had functional bloating. In contrast to a previous placebo-controlled trial of the low FODMAP diet in IBS (Staudacher et al., 2017b), this trial does not support an improvement in abdominal pain in IBD in remission following the
low FODMAP diet, however this requires further investigation since only 38% of participants had IBS (characterised by abdominal pain) at baseline.

This trial suggests that the low FODMAP diet may be more effective in patients with UC compared to CD. This may indicate future potential to direct the diet towards patients most likely to respond to it, thus reducing the number of patients trialling the diet without success. This trial was, however, not designed or powered to detect differences in these sub-groups, therefore future trials are required to confirm these observations.

Several studies have established a relationship between the presence of IBS-like symptoms and poor HR-QOL in patients with IBD in remission (Simrén et al., 2002, Abdalla et al., 2017, Jonefjäll et al., 2016). Patient-perceived control of IBD and HR-QOL were greater following the low FODMAP diet, and FR-QOL was not different between the diets, suggesting that an improvement in GI symptoms may improve HR-QOL without an impairment of FR-QOL. Therefore, the practical challenges associated with following the diet do not appear to counterbalance the benefits of the diet.

5.11.11 Strengths and limitations

This was the first randomised, placebo-controlled trial of the low FODMAP diet in patients with inactive IBD. Patients were selected for this trial based upon stringent criteria and represented a variety of IBD phenotypes, ranging from ileal CD to extensive UC. Furthermore, the inclusion of patients meeting a selection of Rome III functional bowel disorders (IBS, functional bloating, functional diarrhoea) renders the results generalizable to a wide variety of symptom profiles. Meanwhile, the use of a variety of GI symptom measurement tools likely captures the heterogeneous GI symptom profiles of the randomised patients.

Patients were required to fulfil a selection of criteria for inactive IBD for inclusion in the trial. Objective measures of disease activity were used to define remission since the presence of FGS would increase clinical disease activity index scores and would exclude suitable patients. In the only other RCT of the low FODMAP diet in IBD, participants were selected for inclusion based upon a SCCAI score <6 or a HBI score <12, while no objective markers of disease activity were assessed during screening (Pedersen et al., 2017). Therefore, that trial likely included a combination of active IBD and inactive IBD with FGS and it is unclear in which patient group the diet is most likely to be effective.

One limitation of this trial was the measurement of faecal calprotectin during screening. A rapid test was used to quantify faecal calprotectin during screening since this is suited to testing individual samples. A further stool sample was collected at the baseline visit up to 2 weeks later for future faecal calprotectin quantification using ELISA. Due to discrepancy between the rapid
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test and the ELISA and possibly the delay between screening and baseline in some patients, four patients who had faecal calprotectin >250 μg/g at baseline (despite faecal calprotectin <250 μg/g during) were included in the trial. Discrepancy between the rapid tests and ELISA has been observed previously, although it has been suggested that there is better agreement between the tests at lower faecal calprotectin concentrations, such as was expected in this trial (Vestergaard et al., 2008, Wassell et al., 2012, Coorevits et al., 2013).

Prior to this trial, no trials had compared the low FODMAP diet to placebo dietary advice in IBD and the first placebo-controlled trial of the low FODMAP diet in IBS has only recently been published (Staudacher et al., 2017b). The sham dietary advice allows a placebo response to be observed in the control group and permits blinding to diet allocation, in contrast to the only other RCT of the low FODMAP diet in IBD (Pedersen et al., 2017). The sham dietary advice used in the current trial has been piloted and shown to be effective in IBS (Staudacher et al., 2017a), but not in IBD where there may be existing food restrictions and beliefs.

Blinding in the current trial was assessed at end of trial and showed that 74% of participants were able to correctly guess their diet allocation, with more patients in the sham diet capable of identifying diet allocation compared to the low FODMAP diet. This is comparable to previous studies in the research group that have used the same sham dietary advice (unpublished data) but may induce positive or negative expectation bias (Staudacher et al., 2017a). Despite the large proportion of patients capable of identifying diet allocation, placebo response rates of 36% were demonstrated (50-point reduction in the IBS-SSS score in the sham diet group), an unexpected finding had blinding been ineffective. Nonetheless, a study of patient's perceptions of the sham diet may enable modifications to enhance the effectiveness of blinding in this group particularly.

Since the lead researcher provided the dietary advice, it was not possible to be blinded to diet allocation. Although every effort was made to provide dietary advice for both diets within the same length of time and with the same conviction, it is possible that there may have been small, unintentional differences in the delivery of the diets. To provide continuity to patients, the same researcher collected all outcome measures. Non-blinded outcome assessment has been shown to induce observer bias in the measurement of binary and scale outcomes (Hrobjartsson et al., 2012, Hrobjartsson et al., 2013).

To date, there are no GI symptom measures validated specifically for use in patients with IBD with FGS, mandating the use of measures that have not been validated in IBD, such as the IBS-SSS, GSQ and GSRS (Francis et al., 1997). Nonetheless, the GSQ, IBS-SSS and GSRS were able to demonstrate differences in GI symptoms between the groups and non-inflammatory GI
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symptoms in IBD are considered to be ‘functional’ and therefore IBS-specific measures would likely be suitable for use in this patient group. On the other hand, the aforementioned discrepancies between this trial and the previous similarly designed IBS trial may suggest a difference in the validity of the outcome measures between the conditions, which may relate to different symptom experiences and perceptions in IBS and IBD (Staudacher et al., 2017b). A further limitation relating specifically to the IBS-SSS is that it has not been validated in patients with functional bloating and functional diarrhoea, representing 62% of the patients in this trial. Two of the five IBS-SSS questions pertain to abdominal pain, which may not be present in functional bloating and functional diarrhoea and this may have limited the effect size observed in the IBS-SSS.

A major strength of this trial was the measurement of a range of outcomes that may be altered by the low FODMAP diet, including HR-QOL, FR-QOL and IBD-control (Bodger et al., 2013). The IBD-control questionnaire was sensitive to changes in GI symptoms in the current trial, with an improvement in IBD-control in the low FODMAP diet group, further supporting the effectiveness of the diet in IBD.

5.11.12 Conclusion

In conclusion, this trial demonstrated that the low FODMAP diet improves FGS in patients with inactive IBD, but not all GI symptom measures perform equally in reflecting this improvement. Global GI symptoms (adequate relief), bloating, flatulence and stool frequency were lower following the low FODMAP diet compared to the sham diet. In addition, an improvement in perceived control of IBD was apparent following the low FODMAP diet. The impact of the diet on the GI microbiome, immunology and inflammatory markers is unknown. These will be discussed in Chapter 6.
6 Results: the effect of fermentable carbohydrate restriction on the gastrointestinal microbiome, markers of fermentation and peripheral T-cell phenotype in patients with inactive inflammatory bowel disease
6.1 Introduction

Chapter 5 of this thesis demonstrated that the low FODMAP diet improved GI symptoms, HR-QOL and patient-perceived control of IBD, suggesting the low FODMAP diet to be a promising strategy for the management of common FGS in IBD. However, previous trials of the low FODMAP diet in IBS have established that it has a profound effect on the GI microbiome, the most notable and consistent observation being a reduction in immune-modulatory Bifidobacteria (Staudacher et al., 2017b, Staudacher et al., 2012). There is also some evidence that the abundance of *Faecalibacterium prausnitzii*, a species with anti-inflammatory activity that is often depleted in IBD (as discussed in Chapter 1), may be affected by the low FODMAP diet (Halmos et al., 2014a, Hustoft et al., 2017). Changes in immune-modulatory bacteria may influence GI inflammation, therefore an important outcome in this trial was whether the low FODMAP diet exerts an effect on immunology in IBD.

6.2 Aim of this chapter

The aim of this chapter is to report the results of the GI microbiome, faecal short-chain fatty acid (SCFA), faecal pH, inflammatory markers and peripheral T-cell analysis from the randomised, placebo-controlled trial of the low FODMAP diet in patients with inactive IBD, described in Chapter 4.

For all outcomes in this chapter, end of trial values were compared between groups adjusted for baseline values. For metagenomic sequencing only participants providing a stool sample at both baseline and end of trial were included in the analysis. The analysis of the ITT and PP datasets are presented for SCFA and pH.

6.3 Gastrointestinal microbiome: metagenomic sequencing

Forty-two participants provided a stool sample for metagenomic sequencing analysis at both baseline and end of trial (low FODMAP diet, 21, sham diet, 21). An average of 22,690,418 sequencing reads were obtained for each sample. An average of 14,310,652 reads mapped uniquely to the gene catalogue, representing an average of 67% of the reads.

6.3.1 Microbiome composition between groups

6.3.1.1 End of trial comparisons between groups

This section will present the comparisons between the low FODMAP diet and sham diet groups at end of trial, with all analyses adjusted for baseline values. Relative abundance of a given
species is calculated as the average abundance of the 50 marker genes for that species, described in section 4.5.2.5.

6.3.1.1 Alpha diversity and beta diversity

Table 6.1 displays the alpha diversity and beta diversity in the low FODMAP and sham diet groups at end of trial. As can be seen, there was no difference between the low FODMAP diet and sham diet group in bacterial gene richness (P=0.620), species richness (P=0.520) or the Shannon Index (P=0.580) or Simpson index (P=0.450).

Beta diversity was measured using the Bray-Curtis index, where a value of 0 indicates an identical species composition between the samples (low beta-diversity) and a value of 1 indicates no overlap in species composition between the samples (high beta-diversity). The Bray-Curtis index between samples in the low FODMAP diet group was not significantly different to that between samples in the sham diet group (P=0.224).

Table 6.1 Alpha diversity and beta diversity in the diet groups at end of trial

<table>
<thead>
<tr>
<th></th>
<th>Low FODMAP diet (n=21)</th>
<th>Sham diet (n=21)</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>Alpha diversity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacterial gene richness&lt;sup&gt;a&lt;/sup&gt;</td>
<td>498,771 (148,999)</td>
<td>524,660 (184,771)</td>
<td>0.620</td>
</tr>
<tr>
<td>Bacterial species richness&lt;sup&gt;b&lt;/sup&gt;</td>
<td>180 (65)</td>
<td>194 (70)</td>
<td>0.520</td>
</tr>
<tr>
<td>Shannon index</td>
<td>3.52 (0.55)</td>
<td>3.6 (0.7)</td>
<td>0.580</td>
</tr>
<tr>
<td>Simpson index</td>
<td>0.93 (0.05)</td>
<td>0.93 (0.05)</td>
<td>0.450</td>
</tr>
<tr>
<td>Beta diversity</td>
<td></td>
<td></td>
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<tr>
<td>Bray-Curtis index</td>
<td>0.74 (0.03)</td>
<td>0.72 (0.07)</td>
<td>0.224</td>
</tr>
</tbody>
</table>

All values are mean (SD). <sup>a</sup>Bacterial gene count; <sup>b</sup>Metagenomic species count
6.3.1.1.2 Phylum

Figure 6.1 displays the phyla distribution in the diet groups at end of trial. There was no difference in the proportion of the phyla between the diet groups at end of trial (data not shown).

![Phyla distribution diagram]

Figure 6.1 Phyla distribution (proportion of all bacteria) in the low FODMAP and sham diet groups at end of trial

6.3.1.1.3 Species

Of the 616 species that were characterised by metagenomic sequencing, the abundance of 27 species was significantly different at end of trial between the low FODMAP diet and sham diet groups. Figure 6.2 displays the fold difference between the diet groups in the species that were significantly different at end of trial.
Figure 6.2 Fold difference between the low FODMAP diet and sham diet groups in abundance of species with significantly different (P<0.05) abundance at end of trial. Left panel, species with lower abundance in low FODMAP diet group at end of trial; right panel, species with higher abundance in low FODMAP diet group at end of trial. Analysed using a likelihood-ratio test taking into account the effect of diet allocation and baseline values.
The end of trial abundance of bacterial species that have previously shown to be altered by the low FODMAP diet (for example, *Bifidobacterium longum* and *Bifidobacterium adolescentis*, *Adlercreutzia equolifaciens*) or have particular relevance in IBD (for example, *F. prausnitzii*, *Ruminococcus gnavus*) are shown in Table 6.2.

After adjusting for baseline abundance, the relative abundance of Bifidobacteria at the genus level was not significantly differently between the diet groups. The relative abundance of *Faecalibacterium prausnitzii* (all strains), *Bifidobacterium longum*, *Bifidobacterium adolescentis*, *Dorea formicigenerans*, *Dorea longicatena 1*, and unclassified Clostridiales, Prevotella, Firmicutes and Blautia species were significantly lower at end of trial in the low FODMAP diet group compared to the sham diet group (Figure 6.2 and Table 6.2). In contrast, the relative abundance of *Alistipes onderdonkii*, *Hungatella hathewayi*, *Bifidobacterium dentium*, *Anerostipes hadrus*, *Adlercreutzia equolifaciens*, *Dorea longicatena 2*, *Faecalibacterium prausnitzii* SL3/3-M21/2 and unclassified Intestinimonas, Lachnospiraceae and Hungatella species were significantly higher at end of trial in the low FODMAP diet group compared to the sham diet group.
### Table 6.2 Relative abundance of species of interest (either holding importance in IBD or previously shown to reduce following a low FODMAP diet in IBS) at end of trial

<table>
<thead>
<tr>
<th>Genus or species</th>
<th>Low FODMAP diet (n=21)</th>
<th>Sham diet (n=21)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blidobacteria</td>
<td>8.63(^7) (4.41(^7))</td>
<td>3.19(^6) (3.59(^6))</td>
<td>0.073</td>
</tr>
<tr>
<td>B. longum</td>
<td>1.24(^7) (1.81(^7))</td>
<td>6.95(^7) (1.03(^6))</td>
<td>0.006</td>
</tr>
<tr>
<td>B. adolescentis</td>
<td>1.99(^7) (2.78(^7))</td>
<td>2.55(^6) (5.48(^6))</td>
<td>0.005</td>
</tr>
<tr>
<td>F. prausnitzii</td>
<td>1.12(^6) (1.42(^2))</td>
<td>1.65(^6) (1.35(^6))</td>
<td>0.038</td>
</tr>
<tr>
<td>SL3/3-M21/2</td>
<td>1.52(^6) (2.08(^4))</td>
<td>1.35(^6) (1.68(^6))</td>
<td>0.002</td>
</tr>
<tr>
<td>A2-165</td>
<td>2.33(^6) (1.93(^6))</td>
<td>2.81(^6) (2.81(^6))</td>
<td>0.143</td>
</tr>
<tr>
<td>L2-6</td>
<td>3.61(^6) (4.26(^6))</td>
<td>1.30(^6) (1.32(^4))</td>
<td>0.763</td>
</tr>
<tr>
<td>KLE1255</td>
<td>2.68(^6) (3.48(^4))</td>
<td>3.41(^6) (3.89(^4))</td>
<td>0.404</td>
</tr>
<tr>
<td>Adlercreutzia equolifaciens</td>
<td>1.19(^7) (1.85(^7))</td>
<td>6.09(^8) (6.75(^8))</td>
<td>0.018</td>
</tr>
<tr>
<td>Roseburia hominis</td>
<td>4.10(^6) (9.26(^6))</td>
<td>8.48(^7) (1.02(^6))</td>
<td>0.472</td>
</tr>
<tr>
<td>Roseburia intestinalis</td>
<td>4.29(^6) (7.78(^6))</td>
<td>2.58(^6) (6.06(^6))</td>
<td>0.146</td>
</tr>
<tr>
<td>Eubacterium rectale</td>
<td>5.96(^6) (7.22(^6))</td>
<td>5.35(^6) (6.28(^6))</td>
<td>0.823</td>
</tr>
<tr>
<td>Dorea formicigenerans</td>
<td>2.26(^7) (2.46(^7))</td>
<td>2.72(^7) (2.08(^7))</td>
<td>0.021</td>
</tr>
<tr>
<td>Dorea longicatena</td>
<td>2.69(^7) (4.34(^7))</td>
<td>6.22(^7) (7.42(^7))</td>
<td>0.008</td>
</tr>
<tr>
<td>Blautia hansenii</td>
<td>3.84(^9) (1.21(^8))</td>
<td>9.49(^9) (4.35(^9))</td>
<td>0.663</td>
</tr>
<tr>
<td>Blautia hydrogenotrophica</td>
<td>1.97(^9) (7.38(^7))</td>
<td>5.75(^9) (1.33(^8))</td>
<td>0.376</td>
</tr>
<tr>
<td>Bacteroides fragilis</td>
<td>7.12(^7) (1.46(^6))</td>
<td>1.06(^6) (3.19(^6))</td>
<td>0.189</td>
</tr>
<tr>
<td>Bacteroides thetaotaomicron</td>
<td>3.10(^6) (4.20(^6))</td>
<td>2.07(^6) (3.21(^6))</td>
<td>0.485</td>
</tr>
<tr>
<td>Ruminococcus bromii</td>
<td>2.35(^8) (6.12(^8))</td>
<td>1.21(^8) (1.68(^8))</td>
<td>0.765</td>
</tr>
<tr>
<td>Akkermansia muciniphila</td>
<td>6.62(^7) (1.88(^8))</td>
<td>2.09(^6) (6.60(^6))</td>
<td>0.933</td>
</tr>
</tbody>
</table>

All values are mean (SD) relative abundance. *Low FODMAP diet vs. sham diet group at end of trial compared using a likelihood-ratio test taking into account the effect of diet allocation and baseline values.

To establish whether the patients in the low FODMAP and sham diet groups could be distinguished based upon end of trial microbiome composition, a principle component analysis was performed based upon the ratio of the end of trial to baseline abundance of various species (Figure 6.3). This plot shows a separation of the low FODMAP diet and sham diet participants along the vertical axis and corroborates the abundance findings in Table 6.2 that the abundance of certain species, including B. adolescentis, B. longum, Dorea longicatena and F. prausnitzii SL3/3-M21/2 were associated with the sham diet, while other species, including Alistipes onderdonkii and Hungatella hathewayi were associated with the low FODMAP diet.
Figure 6.3 Principle component analysis of species that were significantly different between the low FODMAP diet and sham diet at end of trial. The fold-change of the log10 relative abundance of bacterial species was calculated as the ratio of end of trial to baseline abundance or scores. Blue circles indicate participants on the low FODMAP diet while red circles are participants on the sham diet.

Figure 6.3 Principle component analysis of species that were significantly different between the low FODMAP diet and sham diet at end of trial. The fold-change of the log10 relative abundance of bacterial species was calculated as the ratio of end of trial to baseline abundance or scores. Blue circles indicate participants on the low FODMAP diet while red circles are participants on the sham diet.
6.3.1.4 Bifidobacteria and Faecalibacterium prausnitzii

Bifidobacteria has been consistently shown to decline following a low FODMAP diet in IBS and F. prausnitzii is consistently reduced in IBD with potentially clinical consequences (as described in section 1.1.3.2). These were therefore species of particular interest in this trial.

As can be seen from Figure 6.4 and Table 6.2, the abundance of the Bifidobacteria genus was lower in the low FODMAP diet group than the sham diet group at end of trial, although this did not reach statistical significance (P=0.073). At the species level, the abundance of Bifidobacterium longum and Bifidobacterium adolescentis were significantly lower, and the abundance of Bifidobacterium dentium was significantly higher, in the low FODMAP diet group compared to the sham diet group at end of trial. The abundance of total F. prausnitzii was significantly lower in the low FODMAP diet group compared to the sham diet group at end of trial (P=0.038). At the strain level, F. prausnitzii SL3/3-M21/2 was significantly higher in the low FODMAP diet group at end of trial (see Table 6.2 for data).

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**Figure 6.4** The relative abundance of Bifidobacterium species and Faecalibacterium prausnitzii strains at end of trial in the low FODMAP diet (dark blue bars) and the sham diet group (light blue bars). *P<0.05
6.3.2 Microbiome composition within groups

Due to considerable variability in bacterial differences between diet groups and between individuals, the change in bacterial abundance between baseline and end of trial was analysed separately within the low FODMAP diet group and within the sham diet groups and is presented in this section.

6.3.2.1.1 Alpha diversity and beta diversity

There were no differences in gene richness, species richness, Shannon index, Simpson index or the Bray-Curtis index between baseline and end of trial in either the low FODMAP diet or sham diet groups (data not shown).

6.3.2.1.2 Species

In the low FODMAP diet group, 27 species were significantly different between baseline and end of trial. Species that decreased in abundance between baseline and end of trial included B. longum (7.59^{-7} SD 9.57^{-7} vs. 1.24^{-7} SD 1.81^{-7}, P<0.001), B. pseudocatenulatum (1.94^{-7} SD 4.47^{-7} vs. 3.55^{-8} SD 1.81^{-7}, P=0.036), F. prausnitzii SL3/3-M21/2 (2.30^{-6} SD 2.69^{-6} vs. 1.52^{-6} SD 2.08^{-6}, P=0.029), F. prausnitzii KLE1255 (4.49^{-6} SD 3.33^{-6} vs. 2.68^{-6} SD 3.48^{-6}, P=0.006) (see Figure 6.5 for Bifidobacteria and F. prausnitzii at baseline and end of trial), Dorea longicatena (3.83^{-7} SD 4.98^{-7} vs. 2.69^{-7} SD 4.34^{-7}, P=0.014), and Coprococcus comes (3.17^{-7} SD 3.58^{-7} vs. 2.18^{-7} SD 2.61^{-7}, P=0.028).

Among the species that increased between baseline and end of trial in the low FODMAP diet group were Clostridium citroniae (4.32^{-8} SD 4.65^{-8} vs. 9.11^{-8} SD 1.01^{-7}, P=0.007), Clostridium asparagiforme (4.30^{-8} SD 4.88^{-8} vs. 8.31^{-8} SD 1.09^{-7}, P=0.010), Hungatella hathewayi 2 (1.13^{-8} SD 1.64^{-8} vs. 2.52^{-8} SD 3.07^{-8}, P=0.011), Anaerotruncus colihominis (1.59^{-8} SD 2.51^{-8} vs. 6.56^{-8} SD 1.70^{-7}, P=0.017), Alistipes onderdonkii (2.80^{-6} SD 8.50^{-6} vs. 5.48^{-6} SD 1.70^{-5}, P=0.025), Roseburia hominis (9.08^{-7} SD 1.49^{-6} vs. 4.10^{-6} SD 9.26^{-6}, P=0.035) and Intestinimonas butyriciproducens (4.01^{-8} SD 6.96^{-8} vs. 3.00^{-7} SD 9.03^{-7}, P=0.012).

Only six species were altered between baseline and end of trial in the sham diet group. Species that declined between baseline and end of trial were Coprobiacter fastidiosus (2.34^{-7} SD 4.55^{-7} vs. 1.80^{-7} SD 3.53^{-7}, P=0.022), Sutterella wadsworthenis 1 (6.38^{-8} SD 2.10^{-5} vs. 2.02^{-6} SD 3.49^{-6}, P=0.037), Parabacteroides distasonis (3.28^{-6} SD 4.36^{-6} vs. 2.25^{-6} SD 2.60^{-6}, P=0.045) and Clostridium saccharolyticum (6.83^{-8} SD 1.02^{-7} vs. 4.63^{-8} SD 8.95^{-8}, P=0.045), while an unclassified Blautia (1.62^{-7} SD 1.56^{-7} vs. 2.16^{-7} SD 1.78^{-7}, P=0.009) and an unclassified Clostridiales (3.92^{-9} SD 6.23^{-9} vs. 1.05^{-8} SD 1.81^{-8}, P=0.044) increased.
6.3.2.1.3 Bifidobacteria and Faecalibacterium prausnitzii

The abundance of Bifidobacteria species and *F. prausnitzii* strains at baseline and end of trial is presented in Figure 6.5. As discussed in section 6.3.2.1.2, the abundance of *B. longum*, *B. pseudocatenulatum* and *F. prausnitzii* KLE1255 significantly declined between baseline and end of trial in the low FODMAP diet group. There were no differences in the other Bifidobacteria species and *F. prausnitzii* strains between baseline and end of trial in the low FODMAP diet group, or any of them between baseline and end of trial in the sham diet group.
Figure 6.5 Relative abundance of Bifidobacteria species and Faecalibacterium prausnitizii strains at baseline (dark blue bars) and end of trial (light blue/green bars) in (A) the low FODMAP diet group, (B) the sham diet. *P<0.05
6.3.3 Crohn's disease and ulcerative colitis sub-group analysis

In line with the clinical outcome analysis, sub-group analyses of the GI microbiota in CD (n=19, baseline and end of trial) and UC (n=23, baseline and end of trial) were performed.

At end of trial in patients with CD, there were no differences between the diet groups in gene richness (495,484 SD 185,946 genes vs. 495,451 SD 213,084 genes; P=0.900), species richness (181 SD 80 species vs. 180 SD 79 species; P=0.970), Shannon index (3.4 SD 0.8 vs. 3.5 SD 0.8; P=0.770), Simpson index (0.9 SD 0.1 vs. 0.9 SD 0.1; P=0.446) or beta-diversity (Bray-Curtis Index) (P=0.567). The relative abundance of a limited number of species was significantly different between the low FODMAP diet and sham diet at end of trial in patients with CD, including Adlercreutzia equolifaciens (2.75\(^{-8}\) SD 2.74\(^{-8}\) vs. 5.54\(^{-8}\) SD 6.39\(^{-8}\), P=0.003), Bacteroides xylanisolvens (1.43\(^{-5}\) SD 2.58\(^{-6}\) SD 4.99\(^{-6}\), P=0.008), Eubacterium hallii (5.35\(^{-8}\) SD 6.15\(^{-8}\) vs. 1.73\(^{-7}\) SD 1.57\(^{-7}\), P=0.041), Dorea formicigenerans (1.00\(^{-7}\) SD 6.40\(^{-8}\) vs. 2.02\(^{-7}\) SD 1.86\(^{-7}\), P=0.004), Dorea longicatena (1.19\(^{-7}\) SD 7.84\(^{-8}\) vs. 5.72\(^{-7}\) SD 5.70\(^{-7}\), P=0.001), F. prausnitzii SL3/3-M21/2 (1.87\(^{-6}\) SD 2.39\(^{-6}\) vs. 1.17\(^{-6}\) SD 1.90\(^{-6}\), P=0.033) and F. prausnitzii KLE1255 (1.13\(^{-6}\) SD 8.88\(^{-7}\) vs. 2.48\(^{-6}\) SD 2.89\(^{-6}\), P=0.023).

At end of trial in patients with UC, there were no differences in gene richness (500,795 SD 129,693 genes vs. 556,789 SD 152,407 genes; P=0.400), species richness (180 SD 58 species vs. 209 SD 60 species; P=0.310), Shannon index (3.6 SD 0.4 vs. 3.7 SD 0.6; P=0.640), Simpson index (0.9 SD 0.0 vs. 0.9 SD 0.1; P=0.670) or beta-diversity (Bray-Curtis Index) (P=0.594). The relative abundance of several species was significantly different at end of trial in the low FODMAP group compared to the sham diet group, including B. adolescens (1.52\(^{-7}\) SD 2.65\(^{-7}\) vs. 1.72\(^{-8}\) SD 2.79\(^{-6}\), P=0.014), B. bifidum (8.42\(^{-8}\) SD 1.62\(^{-7}\) vs. 2.50\(^{-7}\) SD 4.20\(^{-7}\), P=0.042), B. longum (1.60\(^{-7}\) SD 2.18\(^{-7}\) vs. 7.21\(^{-7}\) SD 1.13\(^{-6}\), P=0.001), Alistipes onderdonkii (9.11\(^{-7}\) SD 1.25\(^{-6}\) vs. 4.06\(^{-6}\) SD 1.06\(^{-6}\), P=0.018), F. prausnitzii SL3/3-M21/2 (1.30\(^{-6}\) SD 1.93\(^{-6}\) vs. 1.55\(^{-6}\) SD 1.47\(^{-6}\), P=0.008) and Ruminococcus bicirculans (8.78\(^{-7}\) SD 2.18\(^{-6}\) vs. 2.97\(^{-7}\) SD 5.15\(^{-6}\), P=0.005), Hungatella hathewayi 2 (2.50\(^{-8}\) SD 2.60\(^{-8}\) vs. 3.83\(^{-9}\) SD 9.37\(^{-9}\), P=0.034), Dorea longicatena 2 (2.61\(^{-7}\) SD 6.72\(^{-7}\) vs. 8.13\(^{-8}\) SD 1.16\(^{-7}\), P=0.036) and Roseburia intestinalis (5.09\(^{-6}\) SD 8.80\(^{-6}\) vs. 4.71\(^{-6}\) SD 8.35\(^{-6}\), P=0.017).

Figure 6.6 and 6.7 show PCA plots of the log fold-change in abundance (log end of trial abundance divided by baseline abundance) of species that were significantly different between the diets at end of trial, in CD and UC. Figure 6.6 shows that in CD, several bacterial species were associated with the sham diet, including Blautia obeum, Eubacterium hallii and Dorea formicigenerans. Meanwhile, Parabacteroides distasonis and Clostridium citroniae were associated with the low FODMAP diet group. In UC, B. bifidum, B. longum, Roseburia intestinalis and F. prausnitzii SL3/3-M21/2 were associated with the sham diet. Similar to the PCA in all
participants (Figure 6.3), Alistipes onderdonkii was associated with the low FODMAP diet in patients with UC.

Figure 6.6 Principle component analysis of species significantly different between the low FODMAP diet and sham diet at end of trial in patients with CD only. The fold-change of the log10 relative abundance of bacterial species was calculated as the ratio of end of trial to baseline abundance. Blue circles indicate participants on the low FODMAP diet while red circles are participants on the sham diet.
Figure 6.7 Principle component analysis of species significantly different between the low FODMAP diet and sham diet at end of trial in patients with UC only. The fold-change of the log10 relative abundance of bacterial species was calculated as the ratio of end of trial to baseline abundance. Blue circles indicate participants on the low FODMAP diet while red circles are participants on the sham diet.
6.4 Markers of fermentation

6.4.1 Faecal SCFA and pH

Faecal SCFA and pH results are presented in Table 6.3. There was no significant difference in baseline pH or SCFA concentrations between the diet groups.

There was no significant difference in end of trial total faecal SCFA concentration or individual SCFA concentrations in the ITT population. However, there was significantly lower end of trial total SCFA concentration as well as significantly lower acetate concentration at end of trial in the low FODMAP diet group compared to the sham diet group in the PP population only.

Sub-group analysis of CD (n=26; 14 low FODMAP diet, 12 sham diet) and UC (n=26; 13 low FODMAP diet, 13 sham diet) showed significantly lower total SCFA in the low FODMAP diet group than in the sham diet group (418.6 SD 177.8 mg/100g vs. 565.0 SD 177.8 mg/100g, P=0.048) in UC only (Figure 6.8), as well as lower acetate (240.4 SD 101.4 mg/100g vs. 324.9 SD 101.4 mg/100g, P=0.045), butyrate (81.4 SD 26.6 mg/100g vs. 104.0 SD 26.6 mg/100g, P=0.043) and valerate (7.1 SD 4.5 mg/100g vs. 11.4 SD 4.5 mg/100g, P=0.023) concentrations in UC only. In CD, there was a significantly lower isobutyrate concentration in the low FODMAP diet than in the sham diet group at end of trial only (7.9 SD 2.5 mg/100g vs. 10.3 SD 2.9, P=0.035).

There was no significant difference in end of trial faecal pH between the diet groups (Table 6.3), nor was there any difference upon sub-group analyses of CD and UC (data not shown).
Table 6.3 Faecal SCFA (mg per 100g faeces) concentrations and pH in the ITT (n=52) and PP populations (n=43)

<table>
<thead>
<tr>
<th></th>
<th>ITT analysis</th>
<th>PP analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low FODMAP diet</td>
<td>Sham diet</td>
</tr>
<tr>
<td>Total SCFA</td>
<td>(n=27)</td>
<td>(n=25)</td>
</tr>
<tr>
<td></td>
<td>433.9 (164.9)</td>
<td>517.0 (165.1)</td>
</tr>
<tr>
<td>Acetate</td>
<td>251.1 (99.4)</td>
<td>302.4 (99.5)</td>
</tr>
<tr>
<td>Butyrate</td>
<td>71.2 (33.2)</td>
<td>86.7 (33.3)</td>
</tr>
<tr>
<td>Propionate</td>
<td>83.3 (44.8)</td>
<td>100.1 (44.9)</td>
</tr>
<tr>
<td>Valerate</td>
<td>8.2 (3.9)</td>
<td>9.8 (3.9)</td>
</tr>
<tr>
<td>Isobutyrate</td>
<td>7.2 (3.4)</td>
<td>8.6 (3.4)</td>
</tr>
<tr>
<td>Isovalerate</td>
<td>10.8 (4.9)</td>
<td>11.8 (4.9)</td>
</tr>
<tr>
<td>pH</td>
<td>6.53 (0.35)</td>
<td>6.46 (0.35)</td>
</tr>
</tbody>
</table>

All values are mean (SD) compared between groups using ANCOVA with baseline values as a covariate. Estimated mean differences and 95% CI are also presented.
Figure 6.8 SCFA concentrations in UC (left panel) and CD (right panel) at end of trial. *Low FODMAP diet vs. sham diet at end of trial, P<0.05. All values are mean (standard error) compared between groups using ANCOVA with baseline values as a covariate.

To determine whether faecal pH is related to SCFA concentration, a Spearman correlation was performed between end of trial faecal pH and total SCFA concentration. There was a moderate negative correlation between faecal pH and SCFA concentration (r=-0.336, P=0.015). Furthermore, linear regression analysis showed that end of trial faecal pH was predicted by SCFA concentration $F(1,50)=29.0$ (P < 0.001), accounting for 35% of the explained variability in pH.

Linear regression analysis showed that total end of trial SCFA concentration could be predicted by diet allocation $F(1,50)=6.73$ (P=0.012), with diet accounting for 10% of the variability in SCFA concentration. Diet allocation could also predict acetate concentration $F(1,50)=6.69$ (P=0.013), propionate concentration $F(1,50)=4.1$ (P=0.048) and valerate concentration $F(1,50)=4.75$ (P=0.034). Acknowledging that GI transit can influence both SCFA concentrations and pH, end of trial faecal total SCFA concentration and pH were analysed adjusted for end of trial Bristol Stool Form Score in addition to baseline faecal concentrations. These analyses showed no significant difference in SCFA concentration or pH between the diets (data not shown).
6.5 Biomarkers

6.5.1 Faecal calprotectin

Faecal calprotectin was measured to determine eligibility during screening using a rapid point-of-care kit (section 4.6.1.1.1). However, actual baseline and end of trial calprotectin was measured using ELISA (section 4.6.1.1.2) since this is the gold standard method. A comparison of the rapid and ELISA faecal calprotectin assessment can be found in section 7.2.

Stool aliquots were available for 52 participants at baseline and 41 at end of trial. There was no difference in faecal calprotectin between the low FODMAP diet (60.0 SD 87.3) and sham diet group (59.6 SD 74.5) at end of trial (P=0.976). Sub-group analyses showed no differences in faecal calprotectin between the low FODMAP diet group and sham diet group in CD or UC.

6.5.2 C-reactive protein

At screening and end of trial, blood samples were collected for CRP analysis. There was no difference in CRP between the low FODMAP diet (2.0 SD 1.6) and sham diet groups (1.6 SD 1.6) at end of trial (P=0.246).

6.6 Peripheral T cell phenotype

The ITT analysis for peripheral T-cell phenotype is presented below. One participant had no blood sample available at baseline (due to difficulty collecting a blood sample), and for one participant the analysis of the baseline sample was prevented by technical issues. These two participants are not included in the T-cell analysis. Hence, there are 50 end of trial samples included in the ITT analysis (low FODMAP diet, 27, sham diet, 23), except for absolute T-cell counts, for which there are 49 end of trial samples since count beads were omitted from the experiment in error.

6.6.1 Total circulating T-cells

There was no significant difference in the absolute number of CD3+ T-cells at end of trial between the low FODMAP diet and sham diet group (1,268,143 SD 7,798 vs. 1,268,000 SD 7,433 cells/ml⁻¹; P=0.996) (Figure 6.9).
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Figure 6.9 Absolute CD3+ T-cells at end of trial in the low FODMAP diet group (n=27) and sham diet group (n=22). All values are cell counts per ml⁻¹ blood and were compared between groups using ANCOVA with baseline values as a covariate.

When sub-group analyses of CD and UC were performed, there was no significant difference in absolute T-cell counts between the diet groups at end of trial (data not shown).

6.6.2 CD4+ and CD8+ T-cells

There was no significant difference in the proportion of total T-cells that were CD4+ between the low FODMAP diet (66.5% SD 2.8%) and sham diet group (66.6% SD 2.8%) (P=0.893) (Figure 6.10). Likewise, there was no significant difference in the proportion of total T-cells that were CD8+ between the low FODMAP diet (30.6% SD 2.5%) and the sham diet group (30.3% SD 2.5%) (P=0.693). Sub-group analysis of CD and UC revealed no significant differences between the diet groups in CD4+ or CD8+ T-cell proportions (data not shown). However, in patients with CD, there were significantly more CD8+ T-cells in the low FODMAP diet group (520,414 SD 229,397 cells/ml⁻¹) compared to the sham diet group (300,458 SD 244,959 cells/ml⁻¹) (P=0.046).
Figure 6.10 Proportion of CD3+ T-cells expressing CD4 at end of trial in the low FODMAP and sham diet groups. Groups were compared using ANCOVA with baseline values as a covariate.

The proportions of CD4+ and CD8+ T-cells that were naïve (expressing CD45RA) and effector/memory (not expressing CD45RA) were also quantified. Between the low FODMAP diet group and sham diet group at end of trial there was no significant difference in the proportion of CD4+ T-cells (49.6% SD 3.3% vs. 49.8% SD 3.3%, P=0.856) or CD8+ T-cells (68.1% SD 6.4% vs. 68.5% SD 6.4%, P=0.826) that were naïve.

There were no significant differences between the diets in absolute cell counts of naïve or effector/memory CD4+ or CD8+ T-cells at end of trial (data not shown). In line with the increased number of total CD8+ T-cells in the low FODMAP diet group in CD only, there was also significantly more naïve CD8+ T-cells in the low FODMAP diet (368,870 SD 6230 cells/ml) compared to the sham diet group (182,223 SD 8870 cells/ml) (P=0.017). There was however no difference in the number of effector/memory CD8+ T-cells in patients with CD (120,582 SD 2256 vs. 77,193 SD 3012 cells/ml; P=0.108). There was also significantly more naïve CD4+ T-cells in the low FODMAP diet group (573,507 SD 8747 cells/ml) compared to the sham diet group (335,329 SD 12,435 cells/ml) (P=0.041) in patients with CD. There were no significant differences in the proportions of naïve and effector/memory CD4+ and CD8+ T-cells in CD and UC (data not shown).

6.6.3 Vδ2+ T-cells

There was no significant difference in the proportion of CD3+ T-cells expressing the Vδ2 receptor between the low FODMAP diet (3.0% SD 1.2%) and sham diet group (3.2% vs. 1.2%) (P=0.489). There were no significant differences between the diets in absolute counts of Vδ2+ T-cells at
end of trial (data not shown). Sub-group analyses of CD and UC showed no significant differences in the proportions of Vδ2+ T-cells at end of trial between the diet groups (data not shown).

### 6.6.4 T-cells expressing α4β7+ integrin

The proportions of different T-cell subsets expressing α4β7 integrin are displayed in Table 6.4. There were no significant differences between the diet groups in the proportion of T-cell subsets expressing α4β7, except for significantly fewer Vδ2+ T-cells expressing α4β7 at end of trial in the low FODMAP diet group compared to the sham diet group. However, there were no differences in the absolute counts of α4β7+ cells in any of the T-cell subsets between the diet groups at end of trial (Table 6.4).

**Table 6.4 Proportions and absolute cell counts of T-cell subsets expressing α4β7 integrin at end of trial**

<table>
<thead>
<tr>
<th>T-cell Subset</th>
<th>Low FODMAP diet (n=27)</th>
<th>Sham diet (n=23)</th>
<th>Mean difference (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Naïve CD4+</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion (%)</td>
<td>67.1 (15.1)</td>
<td>74.0 (15.1)</td>
<td>-6.9 (-15.5, 1.8)</td>
<td>0.116</td>
</tr>
<tr>
<td>Absolute count</td>
<td>333,815 (209)</td>
<td>279,761 (210)</td>
<td>54,054 (-68,014, 176,122)</td>
<td>0.377</td>
</tr>
<tr>
<td><strong>Effector/memory CD4+</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion (%)</td>
<td>38.7 (6.1)</td>
<td>41.1 (6.1)</td>
<td>-2.5 (-5.9, 1.0)</td>
<td>0.164</td>
</tr>
<tr>
<td>Absolute count</td>
<td>166,034 (84,941)</td>
<td>164,934 (85,426)</td>
<td>1100 (-49,498, 51,697)</td>
<td>0.965</td>
</tr>
<tr>
<td><strong>Naïve CD8+</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion (%)</td>
<td>68.9 (13.1)</td>
<td>74.6 (13.1)</td>
<td>-5.7 (-13.2, 1.8)</td>
<td>0.135</td>
</tr>
<tr>
<td>Absolute count</td>
<td>225,275 (129,155)</td>
<td>172,076 (129,403)</td>
<td>53,199 (-22,241, 128,639)</td>
<td>0.163</td>
</tr>
<tr>
<td><strong>Effector/memory CD8+</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion (%)</td>
<td>63.6 (11.3)</td>
<td>69.9 (11.3)</td>
<td>-6.3 (-12.8, 0.1)</td>
<td>0.054</td>
</tr>
<tr>
<td>Absolute count</td>
<td>81,845 (45,788)</td>
<td>80,040 (45,890)</td>
<td>1805 (-25,264, 28,874)</td>
<td>0.894</td>
</tr>
<tr>
<td><strong>Vδ2+</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion (%)</td>
<td>71.6 (10.5)</td>
<td>79.1 (10.9)</td>
<td>-7.5 (-13.6, -1.4)</td>
<td>0.017</td>
</tr>
<tr>
<td>Absolute count</td>
<td>30,535 (20,249)</td>
<td>31,140 (20,726)</td>
<td>-875 (-12,744, 10,933)</td>
<td>0.377</td>
</tr>
</tbody>
</table>

Data are expressed as mean % (SD) for cell proportions and mean cells per ml⁻¹ blood (SD) for absolute counts. Groups were compared using ANCOVA with baseline values as a covariate.

Two visible populations of α4β7+ cells were observed in the effector/memory CD4+ and CD8+ T-cell scatter plots, one at a lower intensity and one at a higher fluorescence intensity. It is known that T-cells express α4β7 at an intermediate intensity prior to becoming dedicated to gut-homing, therefore an additional gate was constructed around the α4β7+ population at the higher intensity (‘α4β7+ high’), presumably reflecting the ‘true’ α4β7+ gut-tropic cells. There
was no significant difference between the diet groups in the proportion of $\alpha_4\beta_7^+$ high CD4+ or $\alpha_4\beta_7^+$ high CD8+ T-cells at end of trial (Figure 6.11).

Figure 6.11 Proportion of effector/memory CD4+ and CD8+ T-cells that were $\alpha_4\beta_7^+$ high at end of trial

Sub-group analyses of CD and UC showed that in the low FODMAP diet (compared to the sham diet), fewer naïve CD4+ T-cells (58.2% SD 17.0% vs. 79.8% SD 17.0%; $P=0.008$), naïve CD8+ T-cells (62.6% SD 14.8% vs. 76.4% SD 14.8%; $P=0.042$) and effector/memory CD8+ T-cells (59.5% SD 11.2% vs. 70.3% SD 11.2%; $P=0.036$) expressed $\alpha_4\beta_7^+$ at end of trial in patients with CD only. In patients with UC, there were significantly more $\alpha_4\beta_7^+$ high effector/memory CD4+ T-cells at end of trial in the low FODMAP diet group compared to the sham diet group (21.4% SD 2.6% vs. 19.2% SD 2.6%; $P=0.042$). Upon analysis of absolute cell counts in the T-cell sub-sets, there were no differences between the diets in the numbers of cells expressing integrin $\alpha_4\beta_7$ at end of trial (data not shown).

6.7 Discussion

6.7.1 Gut microbiome

In this trial, low FODMAP dietary advice induced significant alterations in GI microbiome composition compared to sham dietary advice. This included a lower abundance of several Bifidobacteria, Dorea and Blautia species, and a higher abundance of Adlercreutzia equolifaciens, Alistipes onderdonkii and Hungatella species at the end of the trial in the low FODMAP diet group compared to the sham diet group. Some of these findings are in line with previous trials of the low FODMAP diet in IBS (Staudacher et al., 2017b, Staudacher et al., 2012, Halmos et al.,...). The significantly lower abundance of Bifidobacterium longum and Bifidobacterium adolescentis following the low FODMAP diet in this trial is consistent with findings of several previous trials of the low FODMAP diet in IBS (Staudacher et al., 2017b, Staudacher et al., 2012, Halmos et al.,...).
2014a, Bennet et al., 2017, Hustoef et al., 2017). This likely relates to the reduced intake of fructans and galacto-oligosaccharides (GOS) associated with the low FODMAP diet, which is supported by the FODMAP intake analysis in Chapter 5. An increase in Bifidobacteria abundance following fructan and GOS supplementation (the so-called prebiotic-effect) suggests that this genus is capable of fermenting these carbohydrates in healthy (Ramirez-Farias et al., 2009, Bouhnik et al., 2004, Holscher et al., 2015), IBS (Azpiroz et al., 2017, Silk et al., 2009) and IBD (Lindsay et al., 2006). This is supported by in vitro analyses and relates to the expression of fructofuranosidases and galactosidases by Bifidobacteria (McLaughlin et al., 2015, Watson et al., 2013). However, variation in the hydrolysis of fructans of differing chain lengths is evident among species and strains; for example, many species can proliferate on oligofructose but fewer can do so on inulin (Rossi et al., 2005). Variations in substrate requirements among Bifidobacterium species may explain why some were not affected by the low FODMAP diet in this trial (including B. animalis and B. breve).

The reduction in Bifidobacteria alongside an improvement in GI symptoms seems paradoxical considering that Bifidobacteria abundance has been shown to correlate negatively with pain frequency (Parkes et al., 2008) and Bifidobacteria supplementation improves FGS in some people with IBS (Whorwell et al., 2006). Therefore, a possible explanation for the lack of response in some of the 48% of participants who did not respond to the diet may be the decline in Bifidobacteria. However, in line with the findings of the current trial, a worsening of bacterial 'dysbiosis', defined using a 'dysbiosis index' that included Bifidobacteria abundance (Casen et al., 2015), has been observed alongside an improvement in GI symptoms following the low FODMAP diet in IBS (Bennet et al., 2017). These changes were seen in low FODMAP diet non-responders as well as responders, suggesting this impact of the diet on Bifidobacteria did not affect its ability to improve GI symptoms.

Anti-inflammatory effects of Bifidobacteria have been demonstrated in vitro and in vivo (Riedel et al., 2006, You and Yaqoob, 2012, Dong et al., 2012). Furthermore, supplementation with a Bifidobacteria-containing probiotic may be effective in inducing remission and may be equally as effective as 5-aminosalicylates for maintaining remission in UC (Derwa et al., 2017). This indicates that Bifidobacteria depletion could theoretically be detrimental in IBD. A large randomised, placebo-controlled trial has established that a Bifidobacteria-containing probiotic supplement maintained Bifidobacteria abundance when taken alongside a low FODMAP diet in patients with IBS (Staudacher et al., 2017b). However, strategies to prevent Bifidobacteria depletion during the low FODMAP diet have not been investigated in IBD specifically, but this supports the need for such research.
Although *Faecalibacterium prausnitzii* strains were not significantly different between the low FODMAP and sham diet groups at end of trial, all strains were reduced between baseline and end of trial in the low FODMAP diet group, with two reaching statistical significance, an effect that was not observed in the sham diet group. In a previous trial of patients with IBS, *F. prausnitzii* declined following a low FODMAP diet and increased following subsequent fructooligosaccharide supplementation, indicating that *F. prausnitzii* is influenced by colonic fructan availability (Hustoft et al., 2017). *F. prausnitzii* is strictly anaerobic and ferments a range of substrates including FODMAP and non-FODMAP carbohydrates (Lopez-Siles et al., 2017), thus it would be expected that substrate utilisation would adapt to those available even during the low FODMAP diet. However as described above, the significant decline in Bifidobacteria and other acetate-producers, such as Blautia, following the low FODMAP diet could indirectly affect *F. prausnitzii* abundance through deprivation of acetate (Heinken et al., 2014). In support of this, difficulties have been reported in establishing an *F. prausnitzii* mono-associated murine model and the need for another bacterium, such as *Bacteroides thetaiotaomicron* or *Escherichia coli*, for successful colonisation, suggesting that *F. prausnitzii* is reliant on metabolic and substrate cross-feeding reactions for growth (Wrzosek et al., 2013, Miquel et al., 2015).

*F. prausnitzii* is an immune-modulatory, butyrogenic bacterium with reduced abundance in IBD. A protective effect of *F. prausnitzii* against experimental colitis has been demonstrated in murine models (Martin et al., 2014, Sokol et al., 2008, Martin et al., 2015) and an inverse correlation between *F. prausnitzii* abundance at surgical resection and recurrence 6 months later is observed in patients with CD (Sokol et al., 2008). These effects are hypothesised to result from the production of metabolites, with a peptide capable of blocking NF-κB recently identified in *F. prausnitzii* supernatant (Quévrain et al., 2015, Miquel et al., 2015).

Therefore, it is feasible that a decline in *F. prausnitzii* could have detrimental clinical ramifications especially on the background of existing depletion. However, in this trial there was no apparent effect of the low FODMAP diet on inflammatory markers or gut-homing T-cells (discussed further later in discussion), suggesting that the decline in immune-modulatory Bifidobacteria and *F. prausnitzii* were not harmful, at least in the short-term. The decline in immune-regulatory bacteria (Bifidobacteria and *F. prausnitzii*) could be counterbalanced by a maintenance of other species capable of regulating immune function. For example, *Roseburia intestinalis* has anti-inflammatory effects *in vitro* and *in vivo* and was not reduced by the low FODMAP diet (Shen et al., 2018). It may also relate to the short-term nature of the diet in this trial. In clinical practice, it is common for patients to follow a low FODMAP diet for longer periods of time, and the effect on the GI microbiota and immunology are undetermined and require investigation.
It is noteworthy that studies of the immune-modulatory effects of *F. prausnitzii* have predominantly investigated the A2-165 strain (Martin et al., 2014, Martin et al., 2015, Miquel et al., 2015), of which the decline during the low FODMAP diet did not reach significance in this trial. The immunological implications of a decline in other *F. prausnitzii* strains (SL3/3-M21/2 and KLE1255) are unclear. Supplementing *F. prausnitzii* to prevent the decline in this species during a low FODMAP diet is not currently possible as the extreme oxygen-sensitivity of this species has complicated efforts to develop an *F. prausnitzii* probiotic, although mechanisms involving dietary vitamins may enable survival in the presence of oxygen (Khan et al., 2012a, Khan et al., 2012b, Khan et al., 2014).

The effect of the low FODMAP diet on Bifidobacteria may have indirect effects on other GI bacteria that are not capable of directly fermenting fructans and GOS. Co-culture experiments have established that upon fermentation of fructans by Bifidobacteria, the resulting acetate, shorter-chain oligo-saccharides and sugars are utilised by butyrogenic bacteria such as *F. prausnitzii*, *Eubacterium hallii*, *Anaerostipes caccae* and *Roseburia intestinalis* to facilitate fermentation of other substrates (El-Semman et al., 2014, Moens et al., 2016, Rios-Covian et al., 2015, Belenguer et al., 2006, Falony et al., 2006). Substrate cross-feeding reactions render growth of certain bacteria (e.g. *F. prausnitzii*) dependent upon the end-products of metabolism of other bacteria (e.g. Bifidobacteria) and explains the ability of a low FODMAP diet to reduce bacteria that are not primary degraders of FODMAPs. Other butyrate-producing bacteria, such as Roseburia species and *Eubacterium rectale*, were not affected by the low FODMAP diet, despite them preferentially fermenting FODMAPs such as oligofructose and inulin (Tamanai-Shacoori et al., 2017). These species may be better equipped to adapt to changes in substrate availability and a possible change in luminal pH than *F. prausnitzii*. Furthermore, species and strain-specific substrate preferences have been observed for Roseburia, suggesting that some strains may be compromised while others may thrive following dietary restrictions (Sheridan et al., 2016).

Besides the observed Bifidobacteria depletion, several other genera and species were affected by the low FODMAP diet in the current trial, including Blautia, Adlercreutzia, Alistipes and Dorea spp. The mechanisms for these alterations are unclear but likely relate to altered colonic substrate availability during the low FODMAP diet. Blautia are strict anaerobes that display species-dependent fermentation of a range of substrates including fructose, mannitol and raffinose and some, such as *Blautia hydrogenotrophica*, utilise hydrogen and carbon dioxide as major energy sources, producing acetate (Liu et al., 2008). Dietary changes, such as wholegrain consumption and resistant starch supplementation, have been shown to influence these genera in healthy volunteers (Martinez et al., 2013), (Ordiz et al., 2015).
Adlercreutzia equolifaciens is a hydrogen-consuming bacterium that was elevated following the low FODMAP diet in the current trial, in line with a previous trial of the low FODMAP diet in IBS (McIntosh et al., 2016). A possible emerging mechanism of the low FODMAP diet is an increased abundance of hydrogen-consuming bacteria. This combination of reduced substrate and increased bacteria that metabolise hydrogen could further reduce hydrogen concentrations in the lumen, thus reducing distension. However, targeted investigations of the effect of increasing or reducing the abundance of Adlercreutzia equolifaciens is required.

Alistipes onderdonkii is a bile-tolerant bacterium that was increased following the low FODMAP diet compared to the sham diet in the current trial. Following a diet based on animal fat and protein, Alistipes abundance increases (David et al., 2014). Since the low FODMAP diet requires restriction of carbohydrate-rich foods such as grains, fruits and vegetables, the increase in a bile-tolerant bacterium may reflect an increase in animal product consumption as alternative foods. However, this is not supported by the RCT nutrient analysis, which revealed reduced protein and fat intake following the low FODMAP diet (section 5.7). Alternatively, the reduction in saccharolytic bacteria such as Bifidobacteria and F. prausnitzii, could simply reduce competition for space and nutrients in the GI tract and therefore enable growth of other species.

Dorea species displayed divergent effects following the trial diets, likely relating to species and strain-dependent carbohydrate utilisation. Dorea formicigenerans and Dorea longicatena 1 were significantly reduced following the low FODMAP diet while Dorea longicatena 2 was increased. Dorea species metabolise a range of substrates including lactose, sorbitol, FOS and inulin to produce hydrogen, carbon dioxide, acetate, lactate and formate (Taras et al., 2002). Since Dorea utilise a range of FODMAP substrates, it is conceivable that some species are susceptible to FODMAP deprivation, which may allow others to thrive.

In the current trial, alpha diversity (measured using the Shannon and Simpson indices) was not different between the diets at end of trial, or between baseline and end of trial in either diet group. This is consistent with a placebo-controlled trial in IBS where alpha diversity was not affected by the low FODMAP diet (Staudacher et al., 2017b). Low bacterial diversity is a consistent feature of the GI microbiota in IBD (Halfvarson et al., 2017), and the lack of effect of the low FODMAP diet on diversity may be explained by different baseline diversity compared to IBS. Alternatively, microbiota composition may shift without altering diversity following the low FODMAP diet. Interestingly, patients classified as responders to the low FODMAP diet in this trial had greater alpha diversity (Shannon Index) than non-responders, the possible explanations for which will be discussed in the final discussion (Chapter 7).
This is the first trial to investigate bacterial gene richness following the low FODMAP diet, although species richness was higher following a low FODMAP diet compared to a high FODMAP diet in patients with IBS (McIntosh et al., 2016). This may relate to the change in substrate availability and subsequent reduction in abundance of the more dominant species', which may facilitate growth of the numerous less dominant species'. Clinical parameters have been associated with bacterial richness, suggesting that this may be a clinically relevant, but often overlooked, microbial parameter. In support of this, high bacterial gene richness was characterised by a greater abundance of putatively anti-inflammatory bacteria, including *F. prausnitzii*, in a study of lean and obese individuals, while low bacterial gene richness was associated with putatively pro-inflammatory bacteria such as Bacteroides and *Ruminococcus gnavus* (Le Chatelier et al., 2013).

There were divergent effects of the low FODMAP diet in CD and UC in this trial, albeit with a reduced sample size upon sub-group analysis. A greater number of species were different between the low FODMAP and sham diets in patients with UC than in patients with CD, which mirrors the greater effect of the diet on GI symptoms (Chapter 5) and faecal SCFA concentration in UC (section 6.4.1). The impact of the low FODMAP diet on *B. adolescentis* and *B. longum* abundance were specific to UC as these species were not different between the groups in CD, although this could be the result of type II errors. This is interesting given that a Bifidobacteria-containing probiotic supplement has been shown to be efficacious for inducing and maintaining remission in UC, while there is limited efficacy in CD (Derwa et al., 2017). The UC-specific effect on Bifidobacteria of the low FODMAP diet warrants further investigation.

The lack of a notable effect of the low FODMAP diet in CD may relate to different baseline microbiome compared to UC, which has been demonstrated in several studies. Crohn’s disease is often characterised by reduced *F. prausnitzii* and increased *Escherichia coli*, and UC with reduced *Roseburia hominis* and increased Clostridiaceae (Sokol et al., 2006, Pascal et al., 2017, Machiels et al., 2013). Reduced *F. prausnitzii* abundance has also been observed in UC (Machiels et al., 2013, Morgan et al., 2012), but dysbiosis may be more pronounced in CD than in UC (Pascal et al., 2017).

The notion that baseline microbiome composition may influence response to treatments targeting or influencing the GI microbiota is supported by a recent fructan supplementation trial in which baseline dietary fibre intake was associated with the microbiome alterations following prebiotic supplementation in healthy volunteers (Healey et al., 2018). Although this has not been replicated in IBD, this provides evidence that GI microbiota composition prior to treatment (in this case, possibly diet-induced) influences treatment-induced alterations in microbiota. The lack of effect of the low FODMAP diet on faecal SCFA concentrations in CD despite a clear effect
in UC lends further support to different microbiota abundance at baseline, perhaps constituting bacteria of a lower SCFA-producing capacity, in line with a greater ‘dysbiosis’ (Pascal et al., 2017). A distinct microbiota profile has also been observed in CD with ileal involvement compared to colonic CD (Morgan et al., 2012, Halfvarson et al., 2017, Naftali et al., 2016), suggesting that patients with ileal or ileocolonic CD (constituting 35% of the total patients in this trial) may also display differential microbial alterations following the diets, thus contributing to the observed differences between CD from UC.

### 6.7.2 Stool SCFA and pH

This trial showed no difference between diet groups in total or individual faecal SCFA at end of trial, although there was a trend towards a lower total SCFA (P=0.080) and acetate (P=0.073) concentration in the low FODMAP diet group, which reached statistical significance in the PP population. Reduced faecal SCFA has not been consistently demonstrated in trials of the low FODMAP diet in IBS (Staudacher et al., 2012, Halmos et al., 2014a). Previous trials in the research group observed significantly lower faecal acetate and butyrate concentrations in patients with IBS following a low FODMAP diet compared to the sham diet, similar to the PP population in the current trial (Wilson et al., 2017).

Reduced faecal SCFA during the low FODMAP diet likely relates to the decline in dietary fermentable substrate. Many GI bacteria generate acetate either through polysaccharide fermentation or de novo from hydrogen and carbon dioxide (for example, *Blautia hydrogenotrophica*) (Louis et al., 2014). The non-significantly lower acetate concentration observed following the low FODMAP diet in this trial may relate, at least in part, to the decline in acetate-producing Bifidobacteria. It may also be explained by changes in GI transit time in patients with CD, in whom a reduced stool frequency was observed on the low FODMAP diet (Lewis and Heaton, 1997a).

It is reassuring that faecal butyrate concentrations were unchanged on the low FODMAP diet compared to the sham diet, since it has putative anti-inflammatory effects, such as promoting differentiation of regulatory T-cells (Smith et al., 2013, Furusawa et al., 2013). Butyrate is generated predominantly by bacteria of the Firmicutes phylum via a range of metabolic pathways. Some major butyrate producers significantly declined between baseline and end of trial in the low FODMAP diet group including *F. prausnitzii* and *Eubacterium* whereas others, such as *Roseburia hominis* and *Intestimonas butyroproducens*, significantly increased. This, as well as the availability of two pathways for butyrate generation, may explain why butyrate concentrations were maintained while acetate declined in the PP population (Louis et al., 2010).
The significant reduction in total SCFA, and specifically acetate, butyrate and valerate following the low FODMAP diet in patients with UC, but not CD, is interesting considering the differential effects that were seen on the GI microbiome and GI symptoms in UC and CD. It is feasible that previous colonic mucosal inflammation could result in a lasting impact upon SCFA absorption in the colon. However, no difference in faecal SCFA concentrations was observed between CD and UC at baseline. Furthermore, 77% of patients with CD in the trial had some colonic disease (ileocolonic or colonic CD), and with this rationale SCFA absorption would be affected in colonic CD in a similar manner to UC. Colonic transit time has an impact on faecal SCFA concentrations and pH, as demonstrated by a reduced faecal SCFA concentration and higher pH following a transit-delaying agent in healthy volunteers (Lewis and Heaton, 1997a). However, transit time does not appear to explain the UC-specific differences in SCFA concentrations at end of trial since the low FODMAP diet did not affect stool frequency or consistency in patients with UC; in fact, there was significantly lower stool frequency following the low FODMAP diet in CD only, where conversely no difference in SCFA was seen at end of trial between the groups.

The quantities of different SCFA produced in the caecum likely depends on the microbiome composition. As discussed, microbiome differences are evident between CD and UC (Pascal et al., 2017) and different species were altered by the low FODMAP diet in CD and UC in this trial. In a previous RCT of the low FODMAP diet in children with IBS, responders to the low FODMAP diet were enriched in bacteria with saccharolytic activity (and thus, SCFA-producing capacity) such as Bacteroides and F. prausnitzii at baseline (Chumpitazi et al., 2015), suggesting that a different baseline microbiome could explain the differential effects of the low FODMAP diet on SCFA observed in UC.

There are potential implications of reduced SCFA in UC, particularly butyrate. Although minimal clinical improvement has been observed following butyrate enema in UC (Hamer et al., 2010, Steinhart et al., 1996), an increase in the IL-10 to IL-12 ratio and reduced nuclear translocation of NF-κB in lamina propria macrophages from patients with UC treated with butyrate suggests anti-inflammatory effects (Luhrs et al., 2002, Hamer et al., 2010). Furthermore, SCFA reduce pH of the GI lumen and this limits pathogen colonisation (Blaut, 2002).

Given that SCFA concentrations were not significantly different between the diet groups at end of trial in the analysis of all participants, it is unsurprising that faecal pH was not different. The association between faecal SCFA concentration and pH was supported by a significant negative correlation. Interestingly, no difference in faecal pH was observed between diet groups despite lower SCFA concentrations in patients with UC on the low FODMAP diet. This may be explained by the fact that colonic pH is determined by the concentration of alkaline metabolites such as ammonia as well as acidic SCFA (Newmark and Lupton, 1990). These metabolites were not
measured in this trial, but a concurrent reduction in alkaline metabolites during the low FODMAP diet may have maintained faecal pH in UC.

### 6.7.3 Biomarkers

In active IBD, gastrointestinal symptom changes alone are insufficient to determine the impact of therapeutic interventions on IBD activity and disease course, since GI symptoms do not consistently correlate with GI inflammation (Peyrin-Biroulet et al., 2015). Biomarkers have recently become central in IBD monitoring (Peyrin-Biroulet et al., 2015) and faecal calprotectin and CRP were measured at baseline and end of trial as non-invasive markers of whether the low FODMAP diet had unexpected effects on inflammation.

Reassuringly, there were no significant differences between the diet groups in faecal calprotectin or CRP at end of trial. In the re-challenge trial described in Chapter 3 of this thesis, faecal calprotectin concentration was significantly elevated between baseline and end of trial (following all three FODMAP challenges and the placebo challenge) in patients with CD only. It is unclear whether this was a result of the background low FODMAP diet, the FODMAP challenges or a coincident fluctuation in GI inflammation, however it does indicate that changes in fermentable carbohydrate intakes may influence GI inflammation. Although faecal calprotectin was unchanged in the current trial, this may not reflect subtle alterations in mucosal immune cell phenotypes. Dendritic cells (DC) may drive abnormal T cell responses to antigen in patients with IBD, potentially through increased pro-inflammatory cytokine secretion (Mann et al., 2014, Ng et al., 2011). The low FODMAP diet could alter mucosal DC and T cell phenotypes via GI microbiome alterations, however obtaining mucosal biopsies was not feasible in this trial. Future research should investigate any GI immune cell changes occurring following the low FODMAP diet.

### 6.7.4 Peripheral T-cell phenotype

Immunology was assessed in this trial on the grounds that the low FODMAP diet has an impact upon immune-regulatory bacteria such as Bifidobacteria in patients with IBS (Staudacher et al., 2017b, Staudacher et al., 2012, Halmos et al., 2014a). Indeed, in this trial Bifidobacteria and *F. prausnitzii* were reduced following the low FODMAP diet, as did potentially immune-modulatory SCFA in UC. The proportion of T-cell subsets expressing the gut-homing integrin α4β7 was measured as an indirect and non-invasive indicator of GI inflammation.

No differences were observed between the groups in total T-cells, the proportion of T-cells that were CD4+ or CD8+, or the proportion of CD4+ and CD8+ T-cells that were antigen naïve or differentiated effector/memory T-cells. There were also no significant differences in the
proximities of T-cell subsets expressing the α4β7 integrin between the diets at end of trial, except for a lower proportion of α4β7-expressing Vδ2 T-cells at end of trial in the low FODMAP diet group.

The lack of effect of the low FODMAP diet on inflammation in this trial is incongruent with a previous trial in which a low FODMAP diet resulted in reduced pro-inflammatory cytokines IL-6 and IL-8 in IBS (Hustoft et al., 2017). However, circulating cytokine concentrations are not specific to the GI tract and could reflect changes in inflammation elsewhere in the body. The inconsistency with the findings of the current trial could therefore relate to differences in overall trial design and specifically immunology analysis.

Similar expression of α4β7 by circulating T-cells in the diet groups at end of trial suggests that, reassuringly, there was no microbiota-induced or direct effect of the low FODMAP diet on gut-homing T-cells during the 4-week trial. However, in clinical practice an 8-12 week FODMAP restriction is commonplace, therefore future studies should establish whether a longer-term FODMAP restriction leads to a detrimental impact upon gut-homing T-cells and GI inflammation. Secondly, the reduction in immune-modulatory bacteria following the diet may be counter-balanced by regulatory mechanisms in the gut designed to limit inflammation, such as an increase in regulatory T-cell differentiation, which can be increased by certain bacteria in vitro (Sun et al., 2015).

Thirdly, the measurement of circulating gut-homing T-cells may not reflect subtle immune aberrations at the mucosal level. For example, probiotic consumption has been shown to alter GI mucosal DC phenotype towards a more anti-inflammatory cytokine profile in UC (Ng et al., 2010). Thus, changes in the GI microbiome following the low FODMAP diet may induce alterations to the mucosal immune compartment, while the number of T-cells homing to the GI tract remains unchanged. It was not possible to obtain mucosal biopsies in this trial, however, future studies should address the immune effects of the low FODMAP diet at the mucosal level.

Fourthly, there are other molecules believed to be involved in T-cell homing to the gut, including CCR9 and GPR15, and measuring these in addition to α4β7 may have afforded a more comprehensive assessment of T-cell gut-homing. However, CCR9 appears to be involved predominantly in T-cell homing to the small intestine, while the role of GPR15 in T-cell homing to the GI tract remains uncertain (Adams and Eksteen, 2006, Fischer et al., 2015). In contrast, α4β7 has a well-established role in T-cell homing to both the small intestine and colon (Berlin et al., 1993). In support of the role of α4β7 for the assessment of changes in GI inflammation in IBD, a shift of β7+ T-cells from the circulation to the mucosa has been observed in patients with active IBD (Hart et al., 2004b, Hart et al., 2004a). Furthermore, a greater proportion of naïve
CD4+ T-cells expressed α4β7 in patients with inactive CD compared to healthy controls (Hedin et al., 2014), and elevated expression of α4β7 has also been observed in UC compared to patients with CD or healthy controls (Fischer et al., 2015).

There was a significantly lower proportion of circulating α4β7+ Vδ2+ T-cells in the low FODMAP diet group at end of trial. Typically, a large proportion of circulating Vδ2+ T-cells express α4β7 (>70%, in agreement with the current trial), and are activated by phosphoantigens that are generated by GI microbiome, suggesting a possible role in GI inflammation (McCarthy et al., 2013, McCarthy et al., 2015). A lower proportion of α4β7+ Vδ2+ T-cells following the low FODMAP diet suggests lower homing of Vδ2+ T-cells to the GI tract. However, the absolute number of α4β7+ Vδ2+ T-cells was not different between the diets at end of trial and the lower proportion of these cells may be explained by a higher number of total Vδ2+ T-cells in the low FODMAP diet group, although this was not significantly different between the groups. A true reduction in α4β7+ Vδ2+ T-cells following the low FODMAP diet could be explained by changes in the GI microbiota, and thus in the levels of phosphoantigen exposure in the GI tract. Phosphoantigens are produced by a wide spectrum of GI bacteria and therefore a shift in the bacterial composition could impact upon Vδ2+ T-cells recruited to the GI tract (Eberl et al., 2003).

A lower proportion of α4β7+ naïve CD4+ T-cells and α4β7+ naïve and effector/memory CD8+ T-cells was observed on the low FODMAP diet in patients with CD. This occurred despite no difference in absolute numbers of naïve or memory T-cells between the diets. A reduced proportion of α4β7+ T-cells on the low FODMAP diet in CD suggests that fewer cells were recruited to the GI tract. Effector/memory T-cells are primed to express α4β7 in the gut-associated lymphoid tissues (GALT) in response to antigen presentation by DC (Hart et al., 2010). Therefore, a reduced proportion of these cells may suggest reduced bacterial antigen sampling and presentation to T-cells in the GALT. In the context of the low FODMAP diet, this could reflect reduced overall bacterial numbers (resulting from less fermentable substrate reaching the colon) or the change in GI microbiome composition. The differences in α4β7+ T-cells in CD and not UC could relate to the observed differential microbial effects of the low FODMAP diet in CD and UC, differences in T-cell gut homing relating to varying disease location between CD and UC, or low-grade GI inflammation in CD not detected by faecal calprotectin upon screening.

6.7.5 Significance of findings

This is the first RCT to establish the effects of low FODMAP dietary advice on the GI microbiota, microbial metabolites and T-cell phenotype in patients with IBD. The diet influences GI microbiota composition in a similar pattern to that seen in IBS, namely the reduction in
Bifidobacteria and *F. prausnitzii*, but with some additional novel findings. A trend towards a lower concentration of SCFA was observed following the low FODMAP diet, reaching significance in the PP population and in patients with UC.

Despite the reduction in immune-modulatory bacteria following the low FODMAP diet, few effects on circulating gut-homing T-cells were observed. Fewer Vδ2 T-cells expressed α4β7 and in CD, fewer naïve CD4+ and CD8+ T-cells, effector/memory CD8+ T-cells expressed α4β7 at end of trial in the low FODMAP diet group. While this may be a chance observation owing to fluctuating immunological parameters in IBD, this may be a true consequence of the microbial alterations or an unidentified effect of the low FODMAP diet. The relevance of these findings is currently unclear and the cause of the reduction in gut-homing integrin expression requires further elucidation.

Overall, although the low FODMAP diet reduced immune-modulatory bacteria and SCFA (in patients with UC), alarming increases in inflammatory markers or gut-homing T-cells were not observed. This suggests that the low FODMAP diet is likely safe in IBD when implemented for a period of up to four weeks, as intended in clinical practice. The reality, however, is that due to patient unwillingness to reintroduce FODMAPs or due to clinical demands, longer-term FODMAP restriction is common (Whelan et al., 2018). It is essential that future research establishes the potential immunological and clinical implications of a prolonged decline in immune-modulatory bacteria, and indeed whether FODMAP reintroduction does recover the abundance of these bacteria.

### 6.7.6 Strengths and limitations

A major strength of this trial pertains to the comprehensive analysis of the GI microbiome using metagenomic sequencing, which is a first-ever in the low FODMAP diet. A detailed discussion of methods for microbiome characterisation can be found in section 4.5.2.1. Next-generation sequencing techniques are more time efficient than methods such as qPCR and FISH because millions of nucleotide bases can be sequenced in a short period of time. Metagenomic sequencing allows greater taxonomic resolution than 16S rRNA sequencing since multiple genes are used to assign taxonomy. Furthermore, it allows identification of species for which 16S rRNA sequences are not known and an assessment of the potential functions of the GI microbiome (though due to time constraints, this was not presented in this thesis). The ion proton sequencing technology used in the current study is more time-efficient than Illumina technology and alerts users to errors early in the sequencing process, thus increasing cost efficiency.

Although metagenomic sequencing is a powerful method for the characterisation of the GI microbiota, it is not without drawbacks. Bias can be introduced through variations in sample
collection, storage, DNA extraction, library preparation and sequencing protocols and despite
efforts to standardise metagenomic studies, different research centres continue to use different
protocols and pipelines (Costea et al., 2017). Furthermore, although databases of known genes
are expanding, the function of many genes arising from metagenomic sequencing studies
remain unknown. A theoretical limitation of metagenomic sequencing over 16S sequencing is
that more data must be generated to obtain the same information. This is because 16S
sequencing targets an individual gene that is amplified prior to sequencing, while in
metagenomic studies, genes of interest must be identified from a large pool of sequencing data
(Thomas et al., 2015). Estimates of species richness and alpha and beta-diversity can be affected
by sequencing depth, defined as the average number of times a particular nucleotide is
represented in a collection of raw sequences (Sims et al., 2014, Finotello et al., 2016). A greater
sequencing depth can also overcome the problem associated with sequencing errors (Sims et
al., 2014). Sequencing depth in this trial (20 million reads generated per sample) was comparable
to previous metagenomics studies (Cotillard et al., 2013, Le Chatelier et al., 2013). Finally,
determining absolute bacterial abundance within a sample is difficult using metagenomic
sequencing, rendering it difficult to make comparisons between studies or to identify whether
total bacterial abundance was altered by the diet.

An intention to treat analysis was not performed on the microbiome data in this trial. This
decision was based upon the expected small effect size of the diet on the GI microbiome and
the relatively small sample size compared to other metagenomic studies (Cotillard et al., 2013,
Le Chatelier et al., 2013). This was felt to be justified since the microbiota was a secondary
outcome in this trial, however this does not reflect the impact the diet would have on the GI
microbiome under clinical conditions in which a proportion of patients discontinue or poorly
comply with the diet.

The ability to compare hundreds of species across the study groups facilitates a less biased
characterisation of the GI microbiome and is an advantage of metagenomic sequencing, but the
vast number of comparisons increases the likelihood of a significant difference occurring by
chance between groups (Bland and Altman, 1995). This can be accounted for by applying a
Bonferroni correction for multiple comparisons. In this trial, however, the number of statistical
tests performed for the microbiome analysis was around 500. Applying a Bonferroni correction
would therefore obscure any real effects of the diet on the microbiome since a p-value of 0.0001
would be required for significance. Indeed, a criticism of the Bonferroni correction is that it is
extremely conservative and increases the likelihood of type II error (albeit while reducing type I
error) (Streiner, 2015). Some of the observed effects of the diet, such as the reduction in several
species of Bifidobacteria and F. prausnitzi abundance, were expected based upon previous
evidence and are unlikely to have occurred by chance. For these reasons, correction for multiple comparisons was not performed and this should be acknowledged in interpreting the microbiome data.

The collection of faecal samples is relatively straight-forward and non-invasive for participants, and likely enhances trial retention. However, there is an inherent limitation that the time between participants voiding the sample and sample processing may vary, as might sample storage conditions. To minimise this variation in this trial, all participants were asked to pass the sample less than two hours before providing to the researcher, and were provided with a standardised stool collection kit. Despite these measures, it is likely that there was some variation but this has recently been shown to have a lesser impact on microbiome composition than variations in DNA extraction protocol (Costea et al., 2017).

Faecal bacterial analysis captures the luminal microbiota but does not provide any information on the mucosa-associated microbiota (MAM). Intra-individual differences between the luminal and mucosal microbiota have been observed in healthy individuals (Ringel et al., 2015, Zoetendal et al., 2002) and patients with IBD (Lavelle et al., 2015) and there may be species that are specific to the MAM and are not captured through faecal analysis. However, the bowel preparation required for colonoscopy or flexible sigmoidoscopy is known to profoundly impact the MAM and introduces bias into microbiota assessment in biopsies (Shobar et al., 2016). Furthermore, colonoscopy is considerably more invasive than faecal sample collection and would have limited recruitment to this trial (Denters et al., 2013). The decision to omit biopsies was therefore justified, but effects of the diet on the MAM may have gone undetected.

Longitudinal gradients in the colonic microbiome have also been observed and certain genera may dominate the proximal and distal colon (Lavelle et al., 2016). Thus, the faecal microbiome is representative of the distal colon but not necessarily the proximal colon, although a small study of patients with UC demonstrated greater variability in microbiota between the mucosa and lumen than that observed longitudinally (Lavelle et al., 2016, Lavelle et al., 2015). Likewise, fermentation and SCFA production is greatest in the caecum and the majority of SCFA are absorbed along the length of the colon (Primec et al., 2017). Thus, SCFA concentrations are highest, and pH is lowest, in the caecum, and this is not captured in faecal samples. Measuring SCFA and pH in vivo is possible but labour-intensive, invasive and costly and was not feasible for the measurement of a secondary outcome in this trial (Boets et al., 2015, Cummings et al., 1987, Peters et al., 1992, Mitchell et al., 1985, Millet et al., 2010, Evans et al., 1988).

Faecal calprotectin concentrations can vary under different physiologic and environmental conditions. In healthy controls, patients with IBD and patients with other conditions, faecal
calprotectin concentration was shown to be similar in several aliquots of the same sample compared to a homogenised portion of the sample, suggesting an even distribution of faecal calprotectin within stools (Røseth et al., 1992). Furthermore, faecal calprotectin is stable in faeces for up to 48 hours at room temperature. Faecal samples in the current trial were collected within 2 hours of evacuation and frozen at -80°C shortly thereafter, therefore storage should not have affected faecal calprotectin concentrations. Significant diurnal and day-to-day variation in faecal calprotectin was observed in a study of patients with active UC (Lasson et al., 2015). However, this variation was most marked in patients with a high faecal calprotectin and patients in the current trial were recruited based upon a faecal calprotectin of <250 μg/g at screening, therefore significant faecal calprotectin fluctuations would not be expected in these patients.

This trial was unique in the measurement of immunology, a parameter omitted from the majority of previous low FODMAP diet trials. An exception is one trial in which a decline in the concentration of serum pro-inflammatory cytokines (IL-6, IL-8) was observed following the low FODMAP diet (Hustoft et al., 2017). Unfortunately, circulating serum cytokines are not indicative of the GI immune state specifically and may be elevated or reduced due to immune alterations anywhere in the body. Therefore, a gut-homing integrin was used in the current trial to assess the proportion of T-cells primed in the GALT to migrate to the GI tract. This indicates the level of antigen-specific T-cell priming occurring in GALT, which could theoretically be influenced by changes to the nature of luminal antigen contacting mucosal DC (Johansson-Lindbom et al., 2005).

The hypothesis in this trial was that a reduction in immune-modulatory bacteria, such as Bifidobacteria and F. prausnitzii, following the low FODMAP diet may influence mucosal DC phenotypes and thus influence the proportion of T-cells primed by DC to express the α4β7 gut-homing integrin (Dudda et al., 2005). However, to date there are no studies definitively proving that changes to GI microbiome composition directly influence T-cell gut-homing integrin expression. Although altered proportions of circulating α4β7+ T-cells have been observed in IBD (Fischer et al., 2015, Hedin et al., 2014), thus justifying the assessment of this marker, circulating T-cell phenotype may not be sufficiently sensitive to reflect subtle mucosal GI immune alterations. Analysing mucosal immune cell numbers and phenotypes might have enhanced the assessment of the immunological impact of the diet but the aforementioned constraints associated with colonoscopy precluded these analyses.

Flow cytometry is an efficient and powerful method allowing the simultaneous assessment of extra- and intracellular markers on individual cells within a sample. A major limitation of flow cytometry is that results are entirely dependent upon the gating strategies used by the investigator (Jahan-Tigh et al., 2012), which is subject to considerable inter-observer variation.
However, this was minimal in this trial since the same observer conducted all analysis (the thesis author, SC). Furthermore, consistent gating strategies were employed for all samples throughout the trial.

### 6.7.7 Conclusion

This trial has established that the low FODMAP diet influences the GI microbiome in patients with IBD, notably with a reduction in immune-modulatory Bifidobacteria and *F. prausnitzii*. Furthermore, in the per protocol population and patients with UC, SCFA concentrations were reduced following the low FODMAP diet, which may represent a detrimental impact of the diet, given the possible effects of certain SCFA on GI homeostasis (Louis et al., 2014). However, the low FODMAP diet did not appear to negatively impact upon GI immune function, with gut-homing T-cell proportions not affected by the diet. The relevance of these findings, and the impact of longer-term FODMAP restriction and FODMAP reintroduction on the GI microbiota warrants further research.
7 Final discussion
7.1 Summary of findings

A large proportion of patients with IBD (~35%) continue to experience GI symptoms that meet the criteria for a functional bowel disorder even during periods of remission (Halpin and Ford, 2012). These functional GI symptoms (FGS) are associated with poor health-related quality of life (HR-QOL) and greater anxiety and depression (Jonefjäll et al., 2016, Jonefjall et al., 2013, Simrén et al., 2002, Gracie et al., 2017) and represent a clinical dilemma since management with anti-inflammatory or immune-modulatory medication is unlikely to be effective. Retrospective (Gearry et al., 2009a) and prospective (Prince et al., 2016) uncontrolled studies and a randomised non-placebo controlled trial (Pedersen et al., 2017) indicate that dietary fermentable carbohydrate restriction (low FODMAP diet) may reduce FGS in patients with IBD in remission. Unfortunately, the difficulty in blinding dietary studies, the large placebo response rate in IBS and the challenge of isolating the effects of specific food components make it difficult to establish from these studies that a low FODMAP diet is effective in patients with inactive IBD.

The aim of this thesis was to explore the role of fermentable carbohydrates in FGS in IBD using a variety of methodological approaches.

Chapter 2 utilised a dietary intake survey to demonstrate that patients with active IBD and inactive IBD with and without FGS have lower intakes of certain fermentable carbohydrates, including galacto-oligosaccharides (GOS) and sorbitol, compared to healthy controls. These findings are unlikely to relate to differences in energy intake since differences were maintained between groups when adjusted for energy intake. Patients with inactive IBD with FGS may also be more nutritionally vulnerable than healthy controls. It is unknown whether the reduced intake of certain FODMAPs reflects FGS induction following consumption, with subsequent dietary avoidance.

Chapter 3 of this thesis therefore described a double-blind, placebo-controlled, crossover re-challenge trial designed to establish whether pure FODMAP challenges induce GI symptoms in patients with inactive IBD, while avoiding the placebo response associated with unblinded or poorly-blinded dietary treatments and the potential confounding posed by other dietary components that may impact GI symptoms. Following a pure fructan challenge, fewer patients reported adequate relief of GI symptoms and the severity of abdominal pain, bloating and flatulence was greater compared to the glucose placebo challenge. This trial established that fructans are capable of inducing GI symptoms, thus indicating a role for FODMAPs in the induction of FGS in inactive IBD. However, the dose of the fructan challenge was twice that of the GOS and sorbitol challenges and the effects of equal doses of the FODMAPs are unknown.
Furthermore, this trial does not establish that the low FODMAP diet is effective for managing FGS in IBD.

Chapter 4 therefore described a RCT designed to compare low FODMAP dietary advice to sham dietary advice in patients with inactive IBD and FGS, thus controlling for the appreciable placebo response rate characteristic of IBS and IBD (Elsenbruch and Enck, 2015).

Chapter 5 demonstrated that significantly more patients reported adequate relief of GI symptoms and lower severity of abdominal bloating and flatulence following the low FODMAP diet compared to sham diet. The improvement in GI symptoms was accompanied by a greater patient-perceived control of IBD and HR-QOL, while FR-QOL was not adversely affected by the diet. The possible limitations associated with blinding in the sham diet in this trial (discussed in section 5.11), should be taken into account when interpreting the findings.

Chapter 6 describes profound effects of the low FODMAP diet on the GI microbiota. In line with trials of the low FODMAP diet in IBS (Staudacher et al., 2017b, Staudacher et al., 2012), Bifidobacterium longum and Bifidobacterium adolescentis and Faecalibacterium prausnitzii were significantly lower following the low FODMAP diet compared to the sham diet. Interestingly, the difference in total faecal SCFA between the diets failed to reach significance, however in patients with UC, total and individual SCFA were significantly lower following the low FODMAP diet compared to sham diet. Despite a reduction in putatively immune-regulatory bacteria, inflammatory markers (faecal calprotectin and CRP) and peripheral T-cell phenotype remained largely unchanged by the diet, suggesting no down-stream effects on GI immune function.

Studies suggest that patients with IBS responding to the low FODMAP diet have a microbial and volatile organic compound (VOC) profile compound distinct from that of non-responders, suggesting it may be possible to predict patients most likely to respond based upon microbiome profiles. Therefore, section 7.2 describes greater microbial alpha diversity that was observed in patients responding to the low FODMAP diet in the RCT described in Chapters 4-6.

### 7.2 Gastrointestinal microbiota of low FODMAP diet responders

A large proportion of patients experience a GI symptom improvement following the low FODMAP diet, however some do not experience a significant improvement. For example, 48% of participants in the low FODMAP diet group in the RCT (Chapters 5) did not report adequate relief. The ability to predict patients most likely to respond to the low FODMAP diet is an attractive concept given the restrictive and complex nature of the diet. The gut microbiome is a potential source of variation in response to the low FODMAP diet and has been shown to vary
between responders and non-responders in previous studies (Chumpitazi et al., 2015, Bennet et al., 2017).

In the RCT described in Chapters 4-6 of this thesis, the baseline microbiome composition was compared between responders to the low FODMAP diet (reporting adequate relief at end of trial; n=14) and non-responders (not reporting adequate relief at end of trial; n=13). There was a higher baseline bacterial gene richness in the responders (549,318 SD 97,434 genes) compared to the non-responders (416,001 SD 202,468 genes), which failed to reach statistical significance (P=0.076) (Figure 7.1), while baseline Shannon index (alpha diversity) was significantly higher in responders (3.7 SD 0.3) compared to non-responders (3.1 SD 0.8) (P=0.023) (Figure 7.1). There was no difference in other measures of microbial diversity, including the Simpson index and the Bray-Curtis index, between responders and non-responders at baseline.

Responders were enriched in several species at baseline, including Senegalimassilia anaerobia, Bacteroides caccae, Parabacteroides merdae, Alistipes onderdonkii, Eubacterium rectale, Blautia obeum, Ruminococcus torques, Dorea formicigenerans, an unclassified Dorea species, Roseburia inulinivorans and Faecalibacterium sp. CAG:82. Fewer species were enriched in non-responders at baseline but included Blautia hansenii, Blautia sp. CAG:257, an unclassified Blautia, Clostridium citroniae and Clostridium saccharolyticum.

Figure 7.2 represents responders’ and non-responders’ microbiome composition at baseline. Responders’ microbiome composition clusters more closely than non-responders, which appear...
more dispersed. The principle components analysis explains 26.6% of the variance in microbiome composition between responders and non-responders but there remains a major overlap between them, suggesting that responders and non-responders could not be completely discriminated based upon microbiome composition.

Figure 7.2 Principle component analysis depicting baseline microbiome composition in low FODMAP diet responders (patients reporting adequate relief at end of trial; yellow triangles) and non-responders (patients not reporting adequate relief at end of trial; blue circles)

The mechanism explaining greater baseline bacterial gene richness (approaching statistical significance) and alpha diversity (statistically significant) in low FODMAP diet responders is currently unknown. Many bacterial genera previously associated with individuals with high bacterial gene richness were those with saccharolytic capacity, such as Faecalibacterium, Butyrivibrio, Ruminococcaceae and Dorea (Le Chatelier et al., 2013). A previous trial reported greater baseline abundance of bacteria with saccharolytic capacity in children responding to the low FODMAP diet (Chumpitazi et al., 2015) and in line with this, several species enriched in low
FODMAP diet responders in this trial had saccharolytic capacity, including *Eubacterium rectale*, *Roseburia inulinivorans* and Faecalibacterium species. One theory could therefore be that the greater bacterial gene richness and diversity reflects greater saccharolytic and metabolic potential of the GI microbiome, which may be associated with intestinal gas production. Therefore, restricting fermentable substrate during the low FODMAP diet may reduce saccharolytic and metabolic activity and increase the likelihood of a response in these individuals due to greater impact on intestinal gas production. The greater GI symptom improvement and a significant reduction in faecal SCFA concentrations in patients with UC, and not in CD, may represent a greater reduction in saccharolytic fermentation during the low FODMAP diet, which may relate to baseline microbiome composition. Although functional metagenomic analysis was not possible within the timeframe for this thesis, future analysis of the carbohydrate-active enzymes encoded by the GI microbiome of responders and non-responders in this trial will help delineate this proposed mechanism of response to the low FODMAP diet.

A distinct baseline microbial profile has previously been observed in patients with IBS responding to the low FODMAP diet (Bennet et al., 2017). However, the species discriminating responders and non-responders were discordant with those in the current trial, which may reflect different microbiota profiles between IBS and IBD, the different methods of microbiota analysis (the previous trial used a ‘dybiosis’ index measuring a limited number of species, compared with full metagenomics sequencing in the current study) and different definitions of responders between the studies. Distinct faecal VOC profiles have also been reported in patients with IBS responding to low FODMAP dietary advice compared to non-responders (Rossi et al., 2018). Since faecal VOC are derived from the GI microbiome and host diet, this provides further evidence that microbial profiles may predispose to a response to the low FODMAP diet, although it is unclear exactly which compounds are associated with response.

Microbiome profiles have also been associated with response to other IBD therapies. In a large RCT of ustekinumab treatment, patients with CD entering remission had a higher baseline alpha diversity and Faecalibacterium and Bacteroides abundance compared to non-responders, bearing a striking similarity to the findings of the current trial, although of course that study was treating active disease, not FGS as in the current study (Chapter 4-6). A negative correlation between baseline alpha diversity and clinical disease activity index was also identified in the ustekinumab trial, suggesting that a lower baseline disease activity may be a confounder (Tedjo et al., 2016).

Although this trial suggests the potential for predicting responders to the low FODMAP diet based upon alpha diversity and the abundance of certain bacterial species, the investigation of microbial profiles in low FODMAP diet responders was exploratory and requires confirmation in
appropriately powered trials. Previous evidence also suggests an association between stool consistency and microbial species richness (Vandeputte et al., 2016), indicating a potential influence of clinical characteristics on this parameter of the GI microbiome that should be considered when interpreting these findings. Further larger-scale studies targeted at identifying microbiome composition, genetic potential and metabolite profiles in low FODMAP diet responders are essential to reduce the number of patients unnecessarily attempting this complex diet.

7.3 Faecal calprotectin assessment

Faecal calprotectin was measured using a rapid point-of-care kit to determine eligibility during patient screening (section 3.4.11.3). However, baseline and end of trial calprotectin was subsequently measured using the gold standard ELISA method (section 4.6.1.1.2).

On final data analysis it was observed that, when measured using ELISA, some patients had baseline faecal calprotectin values exceeding the cut-off for inclusion. Further comparison of the rapid point-of-care kit compared with ELISA showed that on average the rapid test results were 17.5 µg/g lower than the ELISA. The Bland-Altman plot (Figure 7.3) shows that there was a smaller difference (better agreement) between the tests at lower concentrations, while at higher concentrations the rapid test underestimates faecal calprotectin. In line with this finding, four participants (8%) were above the threshold for inclusion (250 µg/g) at the baseline visit, despite a rapid test at screening indicating a faecal calprotectin <250 µg/g.
Figure 7.3 Bland-Altman plot of the FCP concentrations obtained by the rapid test and the ELISA against the difference between the two readings for each individual. The middle dotted horizontal line is the mean difference between the rapid test and ELISA (-17.5 µg/g), while the upper (+156 µg/g) and lower (-191 µg/g) horizontal dashed lines represent the limits of agreement (± 2σ).

This limitation of rapid faecal calprotectin screening tests has been highlighted previously (Rodriguez et al., 2017). Studies show imperfect correlation with ELISA results particularly at higher concentrations (Wassell et al., 2012, Rodriguez et al., 2017), indicating that rapid tests may be inadequate for screening patients for clinical trial eligibility unless the faecal calprotectin threshold for inclusion is low. The discrepancy between the tests at higher faecal calprotectin concentrations may lead to inclusion of participants with faecal calprotectin above the permitted threshold, as was the case in the current study for four patients.

### 7.4 Implications of this thesis for clinical practice

The dietary survey (Chapter 2) suggests that patients with inactive IBD with FGS may manipulate dietary FODMAP intake and therefore this should be assessed prior to advising a low FODMAP diet, especially since intakes of some micronutrients were significantly lower in this group compared to healthy controls, indicating a possible greater risk of inadequate nutrient intakes prior to starting a low FODMAP diet. This further supports the need for careful counselling and monitoring during the diet.

The RCT described in Chapters 4-6 established that, with intensive dietetic guidance, the low FODMAP diet is effective in improving FGS in patients with inactive IBD, although not all GI symptom measures perform equally in representing this improvement. Sub-group analysis suggested that the low FODMAP diet may be more effective in UC, however in clinical practice the diet may still be trialled in patients with inactive CD where indicated.
A profound effect of the low FODMAP diet on GI microbiome composition in IBD, with a similar pattern to that observed in IBS, is evident from the RCT described in Chapters 4-6. The effect of FODMAP reintroduction on GI microbiota composition, and whether this returns bacterial abundance to baseline, remains unknown. Trials of the effects of sequential FODMAP reintroduction on GI symptoms, GI microbiota and immunology in both IBS and IBD are therefore warranted. In patients with IBS, a decline in Bifidobacteria abundance during the low FODMAP diet was prevented through concurrent supplementation with a Bifidobacteria and Lactobacilli-containing supplement (Staudacher et al., 2017b). It remains to be established whether the same would be observed in patients with IBD embarking upon a low FODMAP diet. Patients with IBD may experience difficulties in maintaining nutrient intakes during the low FODMAP diet and vigilant dietary counselling should focus on maintaining overall energy intake as well as micronutrient intakes, with a focus on calcium and iron.

7.5 Recommendations for future research

The case-control study described in Chapter 2 contributes to the investigation of dietary intake in IBD, a field currently characterized by studies featuring inappropriate dietary assessment and heterogeneity in terms of patients recruited and methods used. However, it was not possible to recruit the full target sample size (80 participants per study group) in the timeframe for this thesis and the current findings require confirmation through the analysis of the full sample size once recruited. Furthermore, it was not possible to estimate intake of non-nutrients such as food additives, some of which (particularly emulsifiers) have been implicated in microbiota alterations and intestinal inflammation in murine models (Chassaing et al., 2017, Chassaing et al., 2015). Future studies of dietary intake in IBD should include food additives.

The RCT in Chapters 4-6 established that a 4-week low FODMAP diet reduced FGS in patients with inactive IBD, and although this resulted in shifts in microbiome composition and reduced SCFA in the PP population and patients with UC, it did not appear to adversely affect immunology and GI inflammation. However, in clinical practice it is common for patients to embark upon a longer-term FODMAP restriction and it is likely that the microbiome and SCFA alterations would be exacerbated, which may induce a greater immunological effect. Therefore, the effects of, for example, an 8 or 12-week FODMAP restriction should be investigated.

Given that a large proportion of patients in the sham diet group were able to correctly identify diet allocation, future qualitative research should investigate the predictors of identifying the sham diet. This may allow refinement of the sham diet to include and exclude dietary components that increase blinding effectiveness, while maintaining nutrient and FODMAP intakes. Since no adequately-powered feeding studies of the low FODMAP diet have been
performed in IBD, this would provide proof-of-concept evidence of effectiveness of the diet when followed under optimal conditions.

It is reassuring that the low FODMAP diet did not impact inflammatory markers or circulating T-cell phenotype (Chapter 6). However, as described previously, these outcomes may not be sufficiently sensitive to detect a subtle change in mucosal immunology following the low FODMAP diet. This could precede overt inflammation and an investigation of mucosal DC and T-cell phenotypes following the low FODMAP diet are warranted, although would require mucosal biopsies at baseline and following dietary intervention.

The RCT revealed a greater microbial alpha diversity and enrichment in various species, several with saccharolytic activity, in low FODMAP diet responders compared to non-responders. This contributes to a mounting body of evidence that low FODMAP diet responders may be identified prior to beginning the diet, based upon microbiome composition or the profile of metabolites produced (Chumpitazi et al., 2015, Bennet et al., 2017, Rossi et al., 2018). However, this evidence remains limited to exploratory post-hoc analyses of low FODMAP diet trials and requires adequately-powered trials to establish specifically which species and metabolites are predictive of a response. Volatile organic compounds, derived from the GI microbiome and diet, may be altered in IBD compared to healthy controls and may be different in low FODMAP diet responders compared to non-responders (Ahmed et al., 2016, Rossi et al., 2018). Surplus faecal samples from the RCT will be analysed to establish whether the low FODMAP diet impacts upon VOC profiles or whether distinct profiles are present in low FODMAP diet responders, which could, along with microbial profiles, represent a means of predicting responders and personalising dietetic advice in the future.

Greater efficacy of the low FODMAP diet was observed in UC compared with CD. Interestingly, distinct microbiome alterations were observed following the low FODMAP diet in UC and CD, and faecal SCFA concentrations were significantly reduced following the low FODMAP diet in UC only. These findings suggest that microbiome composition may play a role in the greater responses in UC. The sub-group analysis halves the sample size and reduces statistical power and larger trials are warranted to establish whether this UC-specific effect is replicated.

A major strength of metagenomic sequencing is the ability to investigate the effect of therapies on the functional potential of the GI microbiota, which may not be represented adequately in studies of microbiome species composition (Morgan et al., 2012). Bioinformatics analysis of metagenomic sequencing data is complex and time-intensive and the priority in this thesis was the assessment of microbiome composition. Analysis of the pathways encoded within the GI microbiome may provide further insight into the those that are altered by the low FODMAP diet.
and will clarify whether the saccharolytic potential of the GI microbiota is indeed different in responders compared to non-responders. Assessing the pathways encoded for SCFA generation, such as butyrate synthesis, will also clarify whether a long-term low FODMAP diet could be detrimental.

### 7.6 Conclusion of this doctoral thesis

This thesis establishes that fermentable carbohydrates are reduced in the habitual diet of people with inactive IBD who suffer FGS and that fructans play a role in FGS generation in patients with inactive IBD on the background of a low FODMAP diet. When intensive counselling and support is provided by a dietitian, the low FODMAP diet is an effective therapy for the management of FGS in patients with inactive IBD. Although the low FODMAP diet reduces abundance of immune-modulatory bacteria in patients with inactive IBD, it does not cause alterations to inflammatory markers and immunology in the short-term. Therefore, the low FODMAP diet is a safe and cost-effective therapy for FGS in patients with inactive IBD that could improve HR-QOL in many patients.
8 Appendices
8.1 Re-challenge trial participant information sheet

REC reference Number: 13/LO/1878
Chief Investigator: Professor Kevin Whelan
Principal Investigator: Dr Peter Irving
Co-Investigator: Selina Cox

PARTICIPANT INFORMATION SHEET
FODMAPs as Dietary Triggers of Abdominal Symptoms in Inflammatory Bowel Disease

We would like to invite you to participate in this research project. You should only participate if you want to; choosing not to take part will not disadvantage you in any way. Before you decide whether you want to take part, it is important for you to understand why the research is being done and what your participation will involve. Please take time to read the following information carefully and discuss it with others if you wish.

We are providing you with the following information before you agree to take part, which explains the purpose of this research and what it involves. Please ask any questions you have about the information given and we will do our best to explain and provide any further details you may need.

What is the purpose of the study?

This research study aims to help patients and health care professionals at Guy's and St Thomas' hospitals to learn about the effect of certain dietary carbohydrates (FODMAPs) on gut symptoms experienced by patients with inflammatory bowel disease whilst in remission. FODMAPs are poorly digested in the gut and can increase the water and gas production leading to functional gut symptoms (e.g. irritable bowel syndrome or IBS) in some people. FODMAP rich foods include wheat, onion, garlic, pulses and a few other vegetables and fruit and sugar free gums.

Functional gut symptoms are those experienced during times of inactive disease that are not caused by inflammation and may include abdominal discomfort, abdominal bloating, diarrhoea, wind.

This study is being conducted by a research dietitian from King's College London in collaboration with Guy's and St Thomas' NHS Foundation Trust gastroenterology services. We believe the results of this study will be important for the future treatment and dietary management of functional gut symptoms in patients with inflammatory bowel disease.

Who are we recruiting?

We are looking for people who have been diagnosed with inflammatory bowel disease, and who are attending an outpatient clinic at either Guy's or St Thomas' Hospital as part of their care. Eligible participants will either have been advised in the past to follow a low FODMAP diet for the management of gut symptoms or are eligible for this dietary intervention now. Only those people who have experienced a beneficial effect from the low FODMAP diet are eligible for this study. People with active Crohn's disease or ulcerative colitis will be excluded. Also, people who have taken certain medications recently or who have had medication dose changes, those who have had recent gut surgery or who have other psychiatric or chronic diseases will not be eligible. Pregnant and breastfeeding women will also be excluded.

Do I have to take part?
No. Your participation in this study is voluntary and it is entirely up to you whether you take part. Even if you do decide to take part, you can change your mind at any time and withdraw from the study without giving a reason. If you decide you no longer wish to take part, please inform Selina Cox (contact details below). Any decision not to be involved in the study at any time will not affect the standard of care you receive now or in the future. If you agree to take part, you will be asked whether you are happy to be contacted about participation in future studies. Your participation in this study will not be affected should you choose not to be re-contacted.

What will happen to me if I do take part?

**If you have already received dietary advice for a low FODMAP diet** and have experienced improvement and have control of your gut symptoms as a result, you will be asked to undergo Screening, Test Phase and End of Trial visits (see below).

**If you have not yet received dietary advice for a low FODMAP diet**, then you will be asked to come to the hospital or to King’s College London to be given low FODMAP dietary advice by a dietitian. This is part of normal clinical practice and is the reason you have been referred by your doctor. This advice is not part of the research study. You will be asked to follow the low FODMAP diet for a minimum of two weeks whilst monitoring your gut symptoms until your symptoms are satisfactorily controlled. If the low FODMAP diet does not improve your symptoms you will NOT be eligible for participation in the study. In this case you will have a follow up appointment with the dietitian and will be given advice on what to do next. If the low FODMAP diet improves your symptoms, you will be asked to undergo Screening, Test Phase and End of Trial visits (see below).

**Screening**

If you decide to take part in the study, you will be screened by a researcher to check eligibility for the study and to ensure you meet all the inclusion criteria and none of the exclusion criteria. We will fully explain all the study procedures to you and the consent form will need to be signed prior to starting the study. Once the consent form is signed you will be asked to complete a screening questionnaire with the researcher, including answering some questions about your medical history and gut symptoms. This visit should take no longer than 30 minutes. You will also have a sample of blood taken and will be asked to provide stool sample, which you will collect at home. You will be provided with equipment and full instructions on how to do this.

**Starting the Test Phase**

If your gut symptoms are satisfactorily controlled whilst following a strict low FODMAP diet you will be eligible to take part in the study. At this time, you will be asked to come to the hospital or King’s College London where you will be instructed on the Test Phase of the study. The Test Phase will last a minimum of 4 weeks and you will continue to follow a low FODMAP diet throughout. During this time, you will be asked to take 4 different test drinks containing dietary carbohydrates. You will take a different drink each week once a day for 3 days, followed by a 4-day ‘wash-out’ period, when you continue the low FODMAP diet but don’t take any test drinks. Throughout the 3 test days you will be asked to keep a record of your gut symptoms and bowel habits, and to record when each drink was taken.

The order in which you take the drinks will be randomly assigned and you will not be told which test drink you are taking when. They will be identifiable only by a code. You will be provided with full instructions regarding how and when to take these drinks and when to complete your questionnaires.
Throughout the study you will have telephone support with the researcher who will be able to guide you through the study and answer any queries you have or advise in the case of any adverse events or concerns.

**End of trial**

Once you have completed the Test Phase, you will be invited to a final appointment where you will provide second blood and stool samples. Overall there should be 2-3 visits related to doing this study.

**Expenses**

Reimbursements for expenses (e.g. travel, meals, child-care, compensation for loss of earnings, etc) will not be available for this study.

**What are the possible disadvantages and risks of taking part?**

An ethical review of this study has been carried out. The low FODMAP diet and carbohydrate food supplements have no known clinically adverse effects and have been well tolerated in previous studies. You may experience some gut symptoms like those you have previously experienced when not following the low FODMAP diet. There is no evidence that this will impact in any way on your inflammatory bowel disease activity and/or result in a relapse. Some people might find recording symptoms and/or collecting stool as part of the study embarrassing. You will be provided a toilet insert in which to collect your sample which will make this process easier. If you are concerned in any way, please contact one of the researchers for advice. Some people find having blood taking may be uncomfortable. Every effort will be made to minimise any pain or discomfort during this routine process. The test drinks do not contain anything harmful; they only contain different carbohydrates at levels consumed within the normal diet you were following before the low FODMAP diet. There is no evidence that these can affect your inflammatory bowel disease.

**What are the possible benefits of taking part?**

The results of this study may help to answer scientific questions about whether specific dietary carbohydrates cause functional gut symptoms in inflammatory bowel disease whilst in remission. This may therefore help you and other people in the future, as a diet low in these carbohydrates (the low FODMAP diet) may help improve these symptoms and avoid unnecessary drug treatments that may have unpleasant or adverse side effects. The results will also help you identify which carbohydrates you may be sensitive to and are therefore best avoided or kept to a minimum in your diet.

**What happens at the end of the study?**

At the end of the 4-week Test Phase of the study we will tell you what order you received the test drinks in and will also provide you with advice on how to continue your diet. You may also be booked in for a follow-up dietetic outpatient appointment if you wish.

**What if there is a problem?**

If you have a concern about any aspect of this study, you should speak to Selina Cox (contact details are below). We would not expect you to suffer any harm or injury because of your participation in this study. However, in the unlikely event of you suffering any adverse effects because of participating in this study you can contact King’s College London using the details below for further advice and information and may be compensated through King’s College
London’s ‘No Fault Compensation Scheme’. Professor Kevin Whelan, Professor of Dietetics, Tel: 020 7848 3858, Email: kevin.whelan@kcl.ac.uk.

Will my taking part be kept confidential?

Your participation in the study would be completely confidential. From the beginning of your involvement, you will be given an identification number and only your number, NOT your name, will label your completed questionnaires, diaries and other paperwork. Your data will be kept completely anonymously and confidentially under the UK Data Protection Act 1998 and your name will not appear anywhere in any publication or description of our findings. Personal data and unidentifiable research data will be kept for 10 years on a research database and you may be contacted in the future about follow up studies to this project or ethically approved research studies of a similar nature.

What will happen if I don’t want to carry on with the study?

Taking part in this study is entirely voluntary and your decision will in no way affect your current or future care within this Trust. You will not lose any of your legal or ethical rights. You may withdraw from the study at any time without affecting your routine clinical care and you are not obliged to give reasons. However, if you withdraw because of a side effect please inform the research team. You may be withdrawn from the study, if it is considered in your best interests. If you withdraw from the study, we will destroy all your identifiable samples, but we will need to use the data collected up to your withdrawal.

What will happen to any samples I give?

During the study we will collect stool and blood samples. The samples will have no personal details on them, so your identity will not be recognisable. Portions of the samples may be stored in a secure freezer for up to 10 years. Only members of the research team will have access to them. After they have been used, or at the end of 10 years they will be destroyed.

What will happen to the results of the research study?

It is intended that the results of this study may be published in scientific or medical journals. You will not be identified in any report or publication. When the data from the study has been analysed you will receive a summary report of the results.

Who has reviewed the study?

This study has been checked by an independent group of people, called a Research Ethics Committee to protect your safety, rights, well-being and dignity. This study has been reviewed and given favourable opinion by the NRES Committee: London – Camberwell St Giles.

Who is organising and funding the research?

The study is organised and sponsored by King’s College London (Diabetes and Nutritional Sciences Division, King’s College London) and funded by internal funding. The Chief Investigator is Professor Kevin Whelan and the Principal Investigator is Dr Peter Irving.

If I'd like to participate, what should I do?

If you are interested in taking part or have any further questions or concerns at any time, then please call the contact number or write to the email address below. Participation is entirely voluntary, and it is up to you to decide whether to take part. If you decide to take part, you will be given this information sheet to keep and will be asked to sign a consent form. Remember, if you do decide to take part you are still free to withdraw at any time and without giving a reason.
8.2 Re-challenge trial consent form

CONSENT FORM FOR RESEARCH STUDY

Study Ethics Number: 13/LO/1878
Patient Identification Number for this trial:

Title of Project: FODMAPs as Dietary Triggers of Abdominal Symptoms in Patients with Inflammatory Bowel Disease

Please initial to confirm

I confirm that I have read and understand the information sheet dated ....................... for the above study.

I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

I understand that relevant sections of any of my medical notes and data collected during the study may be looked at by responsible individuals from King’s College London, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.

I agree to the recording and storage of personal and unidentifiable research data for the purposes explained to me. I understand that research information will be anonymous. It will be treated as strictly confidential and handled in accordance with the provisions of the UK Data Protection Act 1998.

I agree to be contacted in the future by King’s College London researchers who would like to invite me to participate in follow up studies to this project, or in future ethically approved studies of a similar nature (please circle one): YES NO

I agree to the collection and storage of blood and stool samples for this study.

I agree to my GP being informed of my participation in the study.

I agree to take part in the above research study.
8.3 Re-challenge trial sample diary day

FUNCTIONAL GUT SYMPTOM & STOOL DIARY: Test Phase

1. Every time you open your bowels today please indicate the type in the box(es) below:

   **BOWEL MOVEMENT 1**
   Please indicate its consistency using the Bristol Stool Chart at the end of this questionnaire. *Please enter a value from 1 to 7.*

   **BOWEL MOVEMENT 2**
   Please indicate its consistency using the Bristol Stool Chart at the end of this questionnaire. *Please enter a value from 1 to 7*

   **BOWEL MOVEMENT 3**
   Please indicate its consistency using the Bristol Stool Chart at the end of this questionnaire. *Please enter a value from 1 to 7*

   **BOWEL MOVEMENT 4**
   Please indicate its consistency using the Bristol Stool Chart at the end of this questionnaire. *Please enter a value from 1 to 7.*

   **BOWEL MOVEMENT 5**
   Please indicate its consistency using the Bristol Stool Chart at the end of this questionnaire. *Please enter a value from 1 to 7.*

   **BOWEL MOVEMENT 6**
   Please indicate its consistency using the Bristol Stool Chart at the end of this questionnaire. *Please enter a value from 1 to 7.*

   If you open your bowels more than three times today, please add details of subsequent stools at the end of this questionnaire.
2. Today, do you currently have satisfactory relief of your gut symptoms? (Circle one)

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
</table>

3. At the end of each day, please rate your symptoms by placing a tick in the box that best describes them. If you do not have this symptom, tick ‘absent’

<table>
<thead>
<tr>
<th>Absent</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>I didn’t have this symptom</td>
<td>I had it a bit but it didn’t bother me much</td>
<td>I had it often, it bothered me quite a bit</td>
<td>I had it a lot, it bothered me a great deal</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>A. Abdominal pain / discomfort</th>
<th>Absent</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>(any kind of pain / discomfort in your abdomen)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Abdominal bloating / distension</th>
<th>Absent</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>(swelling in your stomach or belly)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C. Increased flatulence / passing wind</th>
<th>Absent</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>(release of gas from your bottom)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>D. Belching or burping</th>
<th>Absent</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>(bringing up gas through your mouth)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>E. Stomach / abdominal gurgling</th>
<th>Absent</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>(vibrations or noise in your stomach or belly)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>F. Urgency to open bowels</th>
<th>Absent</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>(urgent need to open your bowels)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>G. Incomplete evacuation</th>
<th>Absent</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>(feeling of inability to pass all stool)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>H. Nausea</th>
<th>Absent</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>(feeling sick, but without vomiting)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>I. Heartburn</th>
<th>Absent</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>(burning / discomfort behind your breastbone)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>J. Acid regurgitation</th>
<th>Absent</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>(taste of sour fluid in your mouth)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>K. Tiredness / lethargy</th>
<th>Absent</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>L. Overall symptoms</th>
<th>Absent</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
</table>
### Bristol Stool Chart

<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1</td>
<td>Separate hard lumps, like nuts (hard to pass)</td>
</tr>
<tr>
<td>Type 2</td>
<td>Sausage-shaped but lumpy</td>
</tr>
<tr>
<td>Type 3</td>
<td>Like a sausage but with cracks on its surface</td>
</tr>
<tr>
<td>Type 4</td>
<td>Like a sausage or snake, smooth and soft</td>
</tr>
<tr>
<td>Type 5</td>
<td>Soft blobs with clear-cut edges (passed easily)</td>
</tr>
<tr>
<td>Type 6</td>
<td>Fluffy pieces with ragged edges, a mushy stool</td>
</tr>
<tr>
<td>Type 7</td>
<td>Watery, no solid pieces. <strong>Entirely Liquid</strong></td>
</tr>
</tbody>
</table>
8.4 Re-challenge trial compliance diary

Please take your test drink as instructed in the MORNING and complete the following questions:

4. Please enter the Test Drink Code in the box (this code can be found on the side of the container provided)

5. Please indicate what time you took your test drink (hr: min)

6. Please indicate whether you took your drink with breakfast, a mid-morning snack or without food (Please circle one)

7. How much of the test drink did you consume? (Please circle one)

   All  More than half  Half of it  Less than half  None
   but not all

Please complete the following questions at the END of each Test Drink Day:

8. How much of the time today did you follow the low FODMAP diet? (Please circle one)

   Never  A quarter of the time (occasionally)  Half of the time  Three quarters of the time (mostly)  Always

9. Have you taken any new medications today or had any changes in dose?

   Yes  No

If yes, please provide details in the box below:

Please include details of any new medications or any medications for which you have had a change in dose:

Please keep all of your test drink containers in the bag provided and bring them with you to your next study visit or clinic appointment with the dietitian.
PARTICIPANT INFORMATION SHEET

(DO YOU WILL BE GIVEN A COPY OF THIS INFORMATION SHEET)

Dietary Interventions for Functional Gut Symptoms in Inflammatory Bowel Disease

We would like to invite you to participate in this research project. You should only participate if you want to; choosing not to take part will not disadvantage you in any way. Before you decide whether you want to take part, it is important for you to understand why the research is being done and what your participation will involve. **Please take time to read the following information carefully** and discuss it with others if you wish.

We are providing you with the following information before you agree to take part, which explains the purpose of this research and what it involves. Please ask any questions you have about the information given and we will do our best to explain and provide any further details you may need.

**What is the purpose of the study?**

This research study aims to help patients, doctors and dietitians at Guy’s and St Thomas’ NHS Foundation Trust and Bart’s Health NHS Trust to learn about the effect of certain foods and drinks on gut symptoms, gut bacteria and inflammation in patients with inactive inflammatory bowel disease.

Functional gut symptoms are those experienced during times of inactive disease that are not caused by inflammation and may include abdominal discomfort, abdominal bloating, diarrhoea and wind.

This study is being conducted as part of a PhD project in collaboration with Guy’s and St Thomas’ NHS Foundation Trust/Bart’s Health NHS Trust and King’s College London. We believe the results of this study will be important for the future treatment and dietary management of functional gut symptoms in patients with inflammatory bowel disease.

**Who are we recruiting?**

We are looking for people with inflammatory bowel disease (either Crohn’s disease or ulcerative colitis), thought to be inactive and who are attending an outpatient clinic at either Guy’s Hospital, St Thomas’ Hospital or The Royal London Hospital as part of their care. Eligible participants will have functional gut symptoms, as described above. People with active disease will be excluded. Also, people who have taken certain medications recently or who have had medication dose changes, those who have had recent or extensive gut surgery or who have other psychiatric or chronic diseases will not be eligible. Pregnant and breastfeeding women will also be excluded.

**Do I have to take part?**

No. Your participation in this study is voluntary and it is entirely up to you whether you take part. Even if you do decide to take part, you can change your mind at any time and withdraw from the study without giving a reason. If you decide you no longer wish to take part, please
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time will not affect the standard of care you receive now or in the future. If you agree to take
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studies. Your participation in this study will not be affected should you choose not to be re-
contacted.

What will happen to me if I do take part?

This study involves following an exclusion diet, and there are two possible diets you may be
asked to follow. One of the diets will reduce specific carbohydrates in the diet, while the other
diet is a ‘placebo’ diet. Both diets require you to make changes to the types of food you eat. For
example, depending upon which diet you are allocated to you will be asked to change your
intake of bread, pasta, rice, potatoes, apples, oranges, berries, broccoli and cucumber. Advice
will be given on how to make these changes to your current diet in line with your food
likes/dislikes and budget.

Screening

If you express interest in taking part in the study, you will be screened by a researcher to check
whether you are eligible to take part and to ensure you meet all the inclusion criteria and none
of the exclusion criteria. We will fully explain all the study procedures to you and the consent
form will need to be signed prior to starting the study. Once the consent form is signed you will
be asked to provide a stool sample and a sample of blood (equivalent to around 1 teaspoon) will
be taken. You will be provided with equipment and full instructions on how to collect the stool
sample. These samples will be taken to check whether there may be any inflammation in your
gut. If the levels are low enough then you may be eligible for the study. This screening visit
should take no longer than 30 minutes.

If you meet all the inclusion and none of the exclusion criteria, you will be given a food and gut
symptom diary to complete for the next 7 days. On the final day of this week, the lead researcher
will contact you by telephone to discuss your symptom diaries and determine whether your level
of symptoms makes you eligible for the study.

Starting the Diet Phase: Baseline visit

If your gut symptoms and stool and blood inflammatory markers meet the criteria for the trial,
you will be eligible to take part. At this time, you will be asked to come for the baseline visit at
the hospital or at King’s College London (whichever you prefer).

At this visit, the following will occur:

The lead researcher will review your food and symptom diaries from the baseline week
Your weight and height will be checked
Your medical and medication history and smoking status will be recorded
You will complete two questionnaires about your quality of life, two questionnaires regarding
your inflammatory bowel disease, and one questionnaire about your gut symptoms
You will be asked to provide a stool sample. You will be provided with full instructions and
equipment to do this
A blood sample will be taken (equivalent to around 2 teaspoons)
You will be instructed on the diet to which you have been asked to follow (see section on “Diets”
below).
This visit should take no longer than one hour.

Diets

At the baseline visit you will be randomly selected to follow one of two different diets:

Intervention diet

Placebo diet

You will not know which diet you have been asked to follow. One diet might help reduce gut symptoms, and the other diet is a ‘placebo diet’. The placebo diet is used as a comparison and is designed to appear to be like the intervention diet. Both diets will require you to alter the types of food you eat – however both diets will allow you to eat a wide range of foods and will allow you to eat the right amount of nutrients. A specialist dietitian who is part of the research team will provide the dietary advice and give you detailed written information about the diet you need to follow. You will be asked to continue the diet for 4 weeks. During this time, you will be telephoned weekly by the lead researcher to guide you through the study and answer any queries you have or advise in the case of adverse events or concerns. You will be asked how much of the time you have followed the diet.

In the final week of the diet, you will complete food and symptom diaries (the same as those completed in the screening week).

End of trial: follow-up visit

You will return to the hospital or King’s College London (whichever you prefer) as soon as possible following completion of the 4-week diet to meet with the lead researcher for the follow-up visit.

At this stage, the following will occur:

The lead researcher will review your food, symptom and stool diary from week 4

Your weight will be checked

You will provide a fresh stool sample. You will be provided with equipment and full instructions on how to do this

You will have a blood sample taken (equivalent to around 3 teaspoons)

You will complete two quality of life questionnaires, two questionnaires regarding your disease activity, and one questionnaire about your gut symptoms.

This visit should take no longer than 40 minutes.

Expenses

Reimbursements for expenses will not be available for this study.

What are the possible disadvantages and risks of taking part?

An ethical review of this study has been carried out. The intervention diet in this study may lead to changes in your gut bacteria, and the aim of this study will answer whether this does happen. However, many patients with inflammatory bowel disease have followed this intervention diet previously and no long-term problems have been reported. The placebo diet has no known negative health effects. There is no evidence that the intervention diet or the placebo diet will affect your inflammatory bowel disease or result in a relapse. Some people might find recording
symptoms and/or collecting stool embarrassing. You will be provided with equipment which will make this process easier. If you are concerned in any way, please contact one of the researchers for advice. Some people find having blood taken may be uncomfortable. Every effort will be made to minimise any pain or discomfort during this routine process.

**What are the possible benefits of taking part?**

The results of this study may help to answer scientific questions about whether specific foods worsen functional gut symptoms in patients with inactive inflammatory bowel disease, and whether there is any effect on the gut bacteria. This may therefore help you and other people in the future, as this type of diet may help improve these symptoms and avoid unnecessary drug treatments that may have unpleasant or adverse side effects.

**What happens at the end of the study?**

At the end of the 4-week diet we will tell you which diet you were following. We will also provide you with advice on how to continue your diet, and if you were in the placebo diet group, you will be offered the intervention diet advice. You may also be booked in for a follow-up dietetic outpatient appointment if you wish.

**What if there is a problem?**

If you have a concern about any aspect of this study you should ask to speak to the researchers who will do their best to answer your questions (Selina Cox 0207 848 4552, selina.cox@kcl.ac.uk). Your inflammatory bowel disease will continue to be monitored by your gastroenterologist.

If you remain unhappy and wish to complain formally, you can do this through the Guy’s and St Thomas’ Patients Advice and Liaison Service (PALS) on 0207 188 8801, pals@gstt.nhs.uk. The PALS team are based in the main entrance on the ground floor at St Thomas’ Hospital and on the ground floor at Guy’s Hospital in the Tower Wing.

In the event that something does go wrong, and you are harmed during the research then you may have grounds for legal action for compensation against King’s College London but you may have to pay your legal costs. King’s College London maintains adequate insurance to cover any liabilities arising from the study.

**Will my taking part be kept confidential?**

Your participation in the study would be completely confidential. From the beginning of your involvement, you will be given an identification number and only your number, NOT your name, will label your completed questionnaires, diaries and other paperwork. Your data will be kept completely anonymously and confidentially under the UK Data Protection Act 1998 and your name will not appear anywhere in any publication or description of our findings. Personal data and unidentifiable research data will be kept for 10 years on a research database and when you consent to this study, you will be asked if we may contact you in the future about follow up studies to this project or ethically approved research studies of a similar nature.

**What will happen if I don’t want to carry on with the study?**

Taking part in this study is entirely voluntary and your decision will in no way affect your current or future care within this Trust. You will not lose any of your legal or ethical rights. You may withdraw from the study at any time without affecting your routine clinical care and you are not obliged to give reasons. However, if you withdraw because of a side effect please inform the research team. You may be withdrawn from the study, if it is considered in your best interests.
If you withdraw from the study, we will destroy all your identifiable samples, but we will need to use the data collected up to your withdrawal.

What will happen to any samples I give?

During the study we will collect stool (to measure bacteria, by-products and other markers) and blood samples (to measure inflammation markers). The samples will have no personal details on them, so your identity will not be recognisable; you will be identifiable only by a code, which only the researchers will have access to. Only members of the research team will have access to your samples. The stool samples and blood samples will be analysed by researchers at King’s College London and their academic partners including Queen Mary University of London (London) and Institut de la Recherche Agronomique (INRA) (Paris). Portions of the samples may be stored in a secure freezer for up to 10 years, as they may be valuable for future research. If we wish to use these remaining samples in future research studies, we will contact you to ask for your consent to do so. After they have been used, or at the end of 10 years your samples will be destroyed.

What will happen to the results of the research study?

It is intended that the results of this study may be published in scientific or medical journals. You will not be identified in any report or publication. When the data from the study has been analysed you will receive a summary report of the results.

Who has reviewed the study?

This study has been checked by an independent group of people, called a Research Ethics Committee to protect your safety, rights, well-being and dignity. This study has been reviewed and given favourable opinion by the NRES Committee: London – Dulwich.

Who is organising and funding the research?

The study is organised and sponsored by King’s College London (Diabetes and Nutritional Sciences Division, King’s College London). The study is funded by internal funding as well as by the Kenneth Rainin Foundation (an inflammatory bowel disease charity). The Chief Investigator is Professor Kevin Whelan and the Principal Investigators are Dr Peter Irving (Guy’s and St Thomas’ NHS Trust) and Dr James Lindsay (Barts Health NHS Trust).

If I'd like to participate, what should I do?

If you are interested in taking part or have any further questions or concerns at any time, then please call the contact number or write to the email address below. Participation is entirely voluntary, and it is up to you to decide whether to take part. If you decide to take part, you will be given this information sheet to keep and will be asked to sign a consent form. Remember, if you do decide to take part you are still free to withdraw at any time and without giving a reason. You may also withdraw any data or information you have already provided up until it is transcribed for use in the final report.
### 8.6 RCT consent form

**Title of project:** Dietary Interventions for Functional Gut Symptoms in Inflammatory Bowel Disease

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>I confirm that I have read the information sheet dated..................... (version............) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.</td>
</tr>
</tbody>
</table>
| 2. | I consent to the following:  
• Three visits at King’s College London, Guy’s or St Thomas’ Hospitals, or The Royal London Hospital  
• Taking part in a four week dietary intervention  
• Complete questionnaires and diaries as described in the information sheet |
| 3. | I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. |
| 4. | I agree that my stool samples can be used in the above study and I have been made aware of how any surplus material will be stored, used and disposed of. |
| 5. | I agree that my blood samples can be used in the above study and I have been made aware of how any surplus material will be stored, used and disposed of. |
| 6. | I understand that my samples may be sent to academic partners outside the United Kingdom for analysis, as outlined in the patient information sheet, but my data will be protected at all times. |
| 7. | I understand that any blood and stool samples that are surplus to this study will be stored securely in an anonymised form, identifiable only by a code that only the researchers will have access to. |
| 8. | I agree that I can be contacted following completion of the study to be asked to give consent for my blood and stool samples to be used in future research studies. |
| 9. | I consent to the processing of my personal information for the purposes explained to me. I understand that such information will be handled in accordance with the terms of the Data Protection Act 1998. |
| 10. | I understand that relevant sections of any of my medical notes and data collected during the study may be looked at by responsible individuals from regulatory authorities or from Guy’s and St Thomas’ NHS Foundation Trust or Barts Health NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. |
| 11. | I agree to my GP being informed of my participation in the study. |
| 12. | I agree to take part in the above study. |
8.7 IBS-SSS

IBS Severity Scoring System (IBS-SSS)

INSTRUCTIONS

This form is designed to enable us to record and monitor the severity of your IBS. It is to be expected that your symptoms might vary over time, so please try and answer the questions based on how you currently feel (i.e. over the last 10 days).

1. For questions where a number of different responses are possible, please circle the response appropriate to you

2. Some questions will require you to write an appropriate response

3. Some questions require you to put a cross on a line which enables us to judge the severity of a particular problem.

For example:

How severe was your pain?

Please put a cross (X) anywhere on the line between 0-100% in order to indicate as accurately as possible the severity of your symptom. This example shows a severity of appropriately 90%

IBS-SSS

1. a) Did you suffer from abdominal (tummy) pain in the last 10 days (please circle)?
   
   Yes  
   No

   b) If yes, how severe was/is your abdominal pain?

   0%  
   100% 
   No not very severe quite severe very severe

   No not very severe quite severe very severe

   No not very severe quite severe very severe

   No not very severe quite severe very severe

   No not very severe quite severe very severe

   No not very severe quite severe very severe
c) Please enter the number of days that you had pain in the last 10 days.

Number of days with pain

2. a) Did you suffer from abdominal distension (bloated, swollen or tight tummy) in the last 10 days (please circle)?

Women please ignore any distension associated with your periods

Yes   No

b) If yes, how severe was/is your abdominal distension/tightness

0%    100%
No    not very severe    quite    severe    very
pain   severe

3. How satisfied were you with your bowel habit in the last 10 days?

0%    100%
very    quite    unhappy    very
happy    happy    unhappy

4. Please indicate with a cross on the line below how much your Irritable Bowel Syndrome was/is affecting or interfering with your life in general over the last 10 days

0%    100%
not at all    not    quite    completely a lot
8.8 UK IBDQ

UK Inflammatory Bowel Disease Questionnaire (UK IBDQ)

1. On how many days over the last two weeks have you had loose runny bowel movements?
   a. None (4)
   b. On one or two days only (3)
   c. On three to seven days (2)
   d. On eight to fourteen days (i.e. more than every other day) (1)

2. On how many days over the last two weeks have you felt tired?
   a. None (4)
   b. On one or two days only (3)
   c. On three to seven days (2)
   d. On eight to fourteen days (i.e. more than every other day) (1)

3. In the last two weeks, have you felt frustrated?
   a. No, not at all (4)
   b. Yes, some of the time (3)
   c. Yes, most of the time (2)
   d. Yes, all the time (1)

4. In the last two weeks, has your bowel condition prevented you from carrying out your work or other normal activities?
   a. No, not at all (4)
   b. Yes, for one or two days (3)
   c. Yes, for three to seven days (2)
   d. Yes, for eight to fourteen days (i.e. more than every other day) (1)

5. On how many days over the last two weeks have you opened your bowel more than three times a day?
   a. None (4)
   b. On one or two days only (3)
   c. On three to seven days (2)
   d. On eight to fourteen days (i.e. more than every other day) (1)

6. On how many days over the last two weeks have you felt full of energy?
   a. None (1)
   b. On one to two days only (2)
   c. On three to seven days (3)
   d. On eight to fourteen days (i.e. more than every other day) (4)

7. In the last two weeks have you been worried about being admitted to hospital because of your bowel problem?
   a. No, not at all (4)
   b. Yes, some of the time (3)
8. In the last two weeks did your bowel condition prevent you from going out socially?
   a. No, not at all (4)
   b. Yes, some of the time (3)
   c. Yes, most of the time (2)
   d. Yes, all the time (1)
   e. Does not apply to me

9. On how many days over the last two weeks have your bowels opened accidentally?
   a. None (4)
   b. On one to two days only (3)
   c. On three to seven days (2)
   d. On eight to fourteen days (i.e. more than every other day) (1)

10. On how many days over the last two weeks have you felt generally unwell?
    a. None (4)
    b. On one to two days only (3)
    c. On three to seven days (2)
    d. On eight to fourteen days (i.e. more than every other day) (1)

11. In the last two weeks have you felt the need to keep close to a toilet?
    a. No, not at all (4)
    b. Yes, some of the time (3)
    c. Yes, most of the time (2)
    d. Yes, all the time (1)

12. In the last two weeks, has your bowel condition affected your leisure or sports activities?
    a. No, not at all (4)
    b. Yes, some of the time (3)
    c. Yes, most of the time (2)
    d. Yes, all the time (1)
    e. Does not apply to me

13. On how many days over the last two weeks have you felt pain in your abdomen?
    a. None (4)
    b. On one to two days only (3)
    c. On three to seven days (2)
    d. On eight to fourteen days (i.e. more than every other day) (1)

14. On how many nights over the last two weeks have you been unable to sleep well (days if you are a shift worker)?
    a. None (4)
b. On one to two nights only (3)
c. On three to seven nights (2)
d. On eight to fourteen nights (i.e. more than every other night) (1)

15. In the last two weeks have you felt depressed?
   a. No, not at all (4)
   b. Yes, some of the time (3)
   c. Yes, most of the time (2)
   d. Yes, all the time (1)

16. In the last two weeks have you had to avoid attending events where there was no toilet close at hand?
   a. No, not at all (4)
   b. Yes, some of the time (3)
   c. Yes, most of the time (2)
   d. Yes, all the time (1)

17. On how many days over the last two weeks have you had a problem with large amounts of wind?
   a. None (4)
   b. On one to two days only (3)
   c. On three to seven days (2)
   d. On eight to fourteen days (i.e. more than every other day) (1)

18. On how many days over the last two weeks have you felt off your food?
   a. None (4)
   b. On one to two days only (3)
   c. On three to seven days (2)
   d. On eight to fourteen days (i.e. more than every other day) (1)

19. Many patients with bowel problems have worries about their illness. How often during the last two weeks have you felt worried?
   a. No, not at all (4)
   b. Yes, some of the time (3)
   c. Yes, most of the time (2)
   d. Yes, all the time (1)

20. On how many days over the last two weeks has your abdomen felt bloated?
   a. None (4)
   b. On one to two days only (3)
   c. On three to seven days (2)
   d. On eight to fourteen days (i.e. more than every other day) (1)

21. In the last two weeks have you felt relaxed?
   a. No, not at all (1)
   b. Yes, some of the time (2)
22. On how many days over the last two weeks have you noticed blood with your bowel movements?
   a. None (4)  
   b. On one to two days only (3)  
   c. On three to seven days (2)  
   d. On eight to fourteen days (i.e. more than every other day) (1)

23. In the last two weeks have you been embarrassed by your bowel problem?
   a. No, not at all (4)  
   b. Yes, some of the time (3)  
   c. Yes, most of the time (2)  
   d. Yes, all the time (1)

24. On how many days over the last two weeks have you wanted to go back to the toilet immediately after you thought you had emptied your bowels?
   a. None (4)  
   b. On one to two days only (3)  
   c. On three to seven days (2)  
   d. On eight to fourteen days (i.e. more than every other day) (1)

25. In the last two weeks have you felt upset?
   a. No, not at all (4)  
   b. Yes, some of the time (3)  
   c. Yes, most of the time (2)  
   d. Yes, all the time (1)

26. On how many days over the last two weeks have you had to rush to the toilet?
   a. None (4)  
   b. On one to two days only (3)  
   c. On three to seven days (2)  
   d. On eight to fourteen days (i.e. more than every other day) (1)

27. In the last two weeks have you felt angry because of your bowel problem?
   a. No, not at all (4)  
   b. Yes, some of the time (3)  
   c. Yes, most of the time (2)  
   d. Yes, all the time (1)

28. In the last two weeks, has your sex life been affected by your bowel problem?
   a. No, not at all (4)  
   b. Yes, some of the time (3)  
   c. Yes, most of the time (2)  
   d. Yes, all the time (1)
e. Does not apply to me

29. On how many days over the last two weeks have you felt sick?
   a. None (4)
   b. On one to two days only (3)
   c. On three to seven days (2)
   d. On eight to fourteen days (i.e. more than every other day) (1)

30. In the last two weeks have you felt irritable?
   a. No, not at all (4)
   b. Yes, some of the time (3)
   c. Yes, most of the time (2)
   d. Yes, all the time (1)

31. In the last two weeks, have you felt lack of sympathy from others?
   a. No, not at all (4)
   b. Yes, some of the time (3)
   c. Yes, most of the time (2)
   d. Yes, all the time (1)

32. In the last two weeks, have you felt happy?
   a. No, not at all (1)
   b. Yes, some of the time (2)
   c. Yes, most of the time (3)
   d. Yes, all of the time (4)
### 8.9 IBD control questionnaire

**Inflammatory Bowel Disease Control Questionnaire (IBD-Control)**

1. **Do you believe that:**
   - a) Your IBD has been well controlled in the past two weeks? Yes (2)  No (0)  Not sure (1)
   - b) Your current treatment is useful in controlling your IBD? Yes (2)  No (0)  Not sure (1)

2. **Over the past two weeks, have your bowel symptoms been getting worse, getting better or not changed?**
   - Better (2)  No change (1)  Worse (0)

3. **In the past 2 weeks, did you:**
   - a) Miss any planned activities because of IBD? (e.g. attending school/college, going to work or a social event) Yes (0)  No (2)  Not sure (1)
   - b) Wake up at night because of symptoms of IBD? Yes (0)  No (2)  Not sure (1)
   - c) Suffer from significant pain or discomfort? Yes (0)  No (2)  Not sure (1)
   - d) Often feel lacking in energy (fatigued)? Yes (0)  No (2)  Not sure (1)
   - e) Feel anxious or depressed because of your IBD? Yes (0)  No (2)  Not sure (1)
   - f) Think you need a change to your treatment? Yes (0)  No (2)  Not sure (1)

4. **At your next clinic visit, would you like to discuss:**
   - a) Alternative types of drug for controlling IBD? Yes (0)  No (2)  Not sure (1)
   - b) Ways to adjust your own treatment? Yes (0)  No (2)  Not sure (1)
   - c) Side effects or difficulties with using your medicines? Yes (0)  No (2)  Not sure (1)
   - d) Symptoms that have developed since your last visit? Yes (0)  No (2)  Not sure (1)

5. **How would you rate the OVERALL control of your IBD in the past two weeks?**
   (please mark a vertical line on the scale below)

   | Worst possible control | Best possible control |
8.10 Harvey-Bradshaw Index and partial Mayo score

The Harvey-Bradshaw Index

Questions (Please check one box per number except for number 5):

<table>
<thead>
<tr>
<th>(1)</th>
<th>General well-being (yesterday)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Very well = 0</td>
</tr>
<tr>
<td></td>
<td>Slightly below par = 1</td>
</tr>
<tr>
<td></td>
<td>Poor = 2</td>
</tr>
<tr>
<td></td>
<td>Very poor = 3</td>
</tr>
<tr>
<td></td>
<td>Terrible = 4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(2)</th>
<th>Abdominal pain (yesterday)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None = 0</td>
</tr>
<tr>
<td></td>
<td>Mild = 1</td>
</tr>
<tr>
<td></td>
<td>Moderate = 2</td>
</tr>
<tr>
<td></td>
<td>Severe = 3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(3)</th>
<th>Number of liquid or soft stools per day (yesterday) =</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>(4)</th>
<th>Abdominal mass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None = 0</td>
</tr>
<tr>
<td></td>
<td>Dubious = 1</td>
</tr>
<tr>
<td></td>
<td>Definite = 2</td>
</tr>
<tr>
<td></td>
<td>Definite and tender = 3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(5)</th>
<th>Complications (check any that apply; score 1 per item except for the first box)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Arthralgia</td>
</tr>
<tr>
<td></td>
<td>Uveitis</td>
</tr>
<tr>
<td></td>
<td>Erythema nodosum</td>
</tr>
<tr>
<td></td>
<td>Aphthous ulcers</td>
</tr>
<tr>
<td></td>
<td>Pyoderma gangrenosum</td>
</tr>
<tr>
<td></td>
<td>Anal fissure</td>
</tr>
<tr>
<td></td>
<td>New fistula</td>
</tr>
<tr>
<td></td>
<td>Abscess</td>
</tr>
</tbody>
</table>

Harvey Bradshaw Index Score = [ ] (Remission = <5)
The Simple Clinical Colitis Activity Index (SCCAI)

**Questions** (Please check the box of the answer that best fits your symptoms over the past week):

<table>
<thead>
<tr>
<th>Question</th>
<th>Options</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Over the past week, what is your average number of bowel movements during the day (not including night time)?</td>
<td>0-3 (score 0), 4-6 (score 1), 7-9 (score 2), &gt;9 (score 3)</td>
<td></td>
</tr>
<tr>
<td>(2) Over the past week, what is your average number of bowel movements during the night?</td>
<td>0 (score 0), 1-3 (score 1), &gt;3 (score 2)</td>
<td></td>
</tr>
<tr>
<td>(3) Over the past week, what has been your urgency of defecation?</td>
<td>No rush (score 0), Hurry (score 1), Immediately (score 2), Incontinence – stool leaked out before you could get to a toilet (score 3)</td>
<td></td>
</tr>
<tr>
<td>(4) Over the past week, what has been the amount and frequency of blood in your stool?</td>
<td>None (score 0), Small traces (score 1), Occasionally obviously bloody (score 2), Usually obviously bloody (score 3)</td>
<td></td>
</tr>
<tr>
<td>(5) General well being</td>
<td>Very well (score 0), Slightly below par (score 1), Poor (score 2), Very poor (score 3), Terrible (score 4)</td>
<td></td>
</tr>
<tr>
<td>(6) During the past week, have you had any of the following?</td>
<td>Pyoderma gangrenosum (oozing ulcers, usually on the leg), Erythema nodosum (red, swollen bumps, on the shin), Uveitis (red, painful eyes), Arthritis (joint pain), None of the above</td>
<td>(Check any that apply; score 1 per item)</td>
</tr>
</tbody>
</table>

**Simple Clinical Colitis Activity Score** = [ ] (Remission = <5)
### 8.11 Food-related quality of life questionnaire

In the past **TWO WEEKS**

<table>
<thead>
<tr>
<th>Question</th>
<th>Strongly Agree (1)</th>
<th>Agree (2)</th>
<th>Neither agree nor disagree (3)</th>
<th>Disagree (4)</th>
<th>Strongly disagree (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I have regretted eating and drinking things that have made my IBD symptoms worse</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>My enjoyment of a particular food or drink has been affected by the knowledge that it might trigger my IBD symptoms</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>My IBD has meant that I have had to leave the table while I am eating to go to the toilet</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>I have not been able to predict how long it will take for my body to respond to something I have had to eat or drink due to my IBD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Certain foods have triggered symptoms of my IBD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>My IBD has meant that I have been nervous that if I eat something I will need to go to the toilet straight away</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>I have avoided having food and drink I know might upset my IBD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>I have felt relaxed about what I can eat and drink despite my IBD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>I have felt in control of what I can eat and drink in relation to my IBD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>I have struggled to eat the way that is best for my IBD because of other commitments during the day</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>I have been frustrated about not knowing how food and drink will react with my IBD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>I have had to concentrate on what I have been eating and drinking because of my IBD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>I have been worried that if I eat I will get symptoms of my IBD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>I have felt the way that I eat and drink for my IBD has affected my day to day life</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>The way I have had to eat for my IBD has restricted my lifestyle</td>
<td>Strongly agree (1)</td>
<td>Agree (2)</td>
<td>Neither agree nor disagree (3)</td>
<td>Disagree (4)</td>
</tr>
<tr>
<td>---</td>
<td>---------------------------------------------------------------</td>
<td>---------------------</td>
<td>-----------</td>
<td>--------------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>15</td>
<td>I have had to concentrate on what food I buy because of my IBD</td>
<td>Strongly agree (1)</td>
<td>Agree (2)</td>
<td>Neither agree nor disagree (3)</td>
<td>Disagree (4)</td>
</tr>
<tr>
<td>16</td>
<td>It has been on my mind how my IBD will be affected by what I eat and drink</td>
<td>Strongly agree (1)</td>
<td>Agree (2)</td>
<td>Neither agree nor disagree (3)</td>
<td>Disagree (4)</td>
</tr>
<tr>
<td>17</td>
<td>My IBD has prevented me from getting full pleasure from the food and I have had</td>
<td>Strongly agree (1)</td>
<td>Agree (2)</td>
<td>Neither agree nor disagree (3)</td>
<td>Disagree (4)</td>
</tr>
<tr>
<td>18</td>
<td>I have felt that I need to know what is in the food I am eating due to my IBD</td>
<td>Strongly agree (1)</td>
<td>Agree (2)</td>
<td>Neither agree nor disagree (3)</td>
<td>Disagree (4)</td>
</tr>
<tr>
<td>19</td>
<td>I have felt that I have to be careful about when I have eaten because of my IBD</td>
<td>Strongly agree (1)</td>
<td>Agree (2)</td>
<td>Neither agree nor disagree (3)</td>
<td>Disagree (4)</td>
</tr>
<tr>
<td>20</td>
<td>I have had to be aware of what I am eating due to my IBD</td>
<td>Strongly agree (1)</td>
<td>Agree (2)</td>
<td>Neither agree nor disagree (3)</td>
<td>Disagree (4)</td>
</tr>
<tr>
<td>21</td>
<td>I've missed being able to eat or drink whatever I want because of my IBD</td>
<td>Strongly agree (1)</td>
<td>Agree (2)</td>
<td>Neither agree nor disagree (3)</td>
<td>Disagree (4)</td>
</tr>
<tr>
<td>22</td>
<td>I have felt that I would like to be able to eat and drink like everyone else</td>
<td>Strongly agree (1)</td>
<td>Agree (2)</td>
<td>Neither agree nor disagree (3)</td>
<td>Disagree (4)</td>
</tr>
<tr>
<td>23</td>
<td>I have been happy to eat and drink around people I do not know despite my IBD</td>
<td>Strongly agree (1)</td>
<td>Agree (2)</td>
<td>Neither agree nor disagree (3)</td>
<td>Disagree (4)</td>
</tr>
<tr>
<td>24</td>
<td>I have felt that I have been eating and drinking normally despite my IBD</td>
<td>Strongly agree (1)</td>
<td>Agree (2)</td>
<td>Neither agree nor disagree (3)</td>
<td>Disagree (4)</td>
</tr>
<tr>
<td>25</td>
<td>I have found it hard not knowing if a certain food will trigger IBD symptoms</td>
<td>Strongly agree (1)</td>
<td>Agree (2)</td>
<td>Neither agree nor disagree (3)</td>
<td>Disagree (4)</td>
</tr>
<tr>
<td>26</td>
<td>My IBD has meant I have had to make an effort to get all the nutrients my body needs</td>
<td>Strongly agree (1)</td>
<td>Agree (2)</td>
<td>Neither agree nor disagree (3)</td>
<td>Disagree (4)</td>
</tr>
<tr>
<td>27</td>
<td>I have felt that I haven't known how my IBD will react to food or drink</td>
<td>Strongly agree (1)</td>
<td>Agree (2)</td>
<td>Neither agree nor disagree (3)</td>
<td>Disagree (4)</td>
</tr>
<tr>
<td>28</td>
<td>My IBD has meant that I have had to work hard to fit my eating habits in around my activities during the day</td>
<td>Strongly agree (1)</td>
<td>Agree (2)</td>
<td>Neither agree nor disagree (3)</td>
<td>Disagree (4)</td>
</tr>
</tbody>
</table>

### 8.12 Diet acceptability questionnaire

Please circle the answer most applicable to you.

While following the research diet:

1. **Meal preparation was**

<table>
<thead>
<tr>
<th>Rating</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>-3</td>
<td>Much More Difficult</td>
</tr>
<tr>
<td>-2</td>
<td>More Difficult</td>
</tr>
<tr>
<td>-1</td>
<td>Slightly More Difficult</td>
</tr>
<tr>
<td>0</td>
<td>No Different</td>
</tr>
<tr>
<td>+1</td>
<td>Slightly Easier</td>
</tr>
<tr>
<td>+2</td>
<td>Easier</td>
</tr>
<tr>
<td>+3</td>
<td>Much Easier</td>
</tr>
</tbody>
</table>

2. **The time I spent shopping for food was**

<table>
<thead>
<tr>
<th>Rating</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>-3</td>
<td>Much Longer</td>
</tr>
<tr>
<td>-2</td>
<td>Longer</td>
</tr>
<tr>
<td>-1</td>
<td>Slightly Longer</td>
</tr>
<tr>
<td>0</td>
<td>No Different</td>
</tr>
<tr>
<td>+1</td>
<td>Slightly Shorter</td>
</tr>
<tr>
<td>+2</td>
<td>Shorter</td>
</tr>
<tr>
<td>+3</td>
<td>Much Shorter</td>
</tr>
</tbody>
</table>

3. **The time I spent preparing and cooking meals and snacks was**

<table>
<thead>
<tr>
<th>Rating</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>-3</td>
<td>Much Longer</td>
</tr>
<tr>
<td>-2</td>
<td>Longer</td>
</tr>
<tr>
<td>-1</td>
<td>Slightly Longer</td>
</tr>
<tr>
<td>0</td>
<td>No Different</td>
</tr>
<tr>
<td>+1</td>
<td>Slightly Shorter</td>
</tr>
<tr>
<td>+2</td>
<td>Shorter</td>
</tr>
<tr>
<td>+3</td>
<td>Much Shorter</td>
</tr>
</tbody>
</table>

4. **Finding suitable food choices when eating out was**

<table>
<thead>
<tr>
<th>Rating</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>-3</td>
<td>Much More Difficult</td>
</tr>
<tr>
<td>-2</td>
<td>More Difficult</td>
</tr>
<tr>
<td>-1</td>
<td>Slightly More Difficult</td>
</tr>
<tr>
<td>0</td>
<td>No Different</td>
</tr>
<tr>
<td>+1</td>
<td>Slightly Easier</td>
</tr>
<tr>
<td>+2</td>
<td>Easier</td>
</tr>
<tr>
<td>+3</td>
<td>Much Easier</td>
</tr>
</tbody>
</table>

5. **I found the flavour of the meals and snacks to be**

<table>
<thead>
<tr>
<th>Rating</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>-3</td>
<td>Much Less Appealing</td>
</tr>
<tr>
<td>-2</td>
<td>Less Appealing</td>
</tr>
<tr>
<td>-1</td>
<td>Slightly Less Appealing</td>
</tr>
<tr>
<td>0</td>
<td>No Different</td>
</tr>
<tr>
<td>+1</td>
<td>Slightly More Appealing</td>
</tr>
<tr>
<td>+2</td>
<td>More Appealing</td>
</tr>
<tr>
<td>+3</td>
<td>Much More Appealing</td>
</tr>
</tbody>
</table>
10. Did any benefits of being involved in this study outweigh the burden of being on this diet? (Please circle)

   Yes
   No

11. Overall did you have to make many changes to your diet? (Please circle)

   Yes
   No

12. Was there a food or drink that you missed a lot? (Please circle)

   Yes
   No

13. If yes, what food or drink was it?

   ____
8.13 Example pages of diet resources

8.13.1 Low FODMAP diet booklet
### 8.13.2 Sham diet booklet

<table>
<thead>
<tr>
<th>Suitable foods: starchy foods</th>
<th>Foods to avoid: starchy foods</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cereal grains &amp; other starchy foods</strong></td>
<td><strong>Cereal grains</strong></td>
</tr>
<tr>
<td>Wheat (e.g. cous cous, bulghur wheat, breads and breakfast cereals listed), potato, sweet potato, polenta, quinoa, barley, amaranth</td>
<td>Rice</td>
</tr>
<tr>
<td><strong>Bread</strong></td>
<td>Oats</td>
</tr>
<tr>
<td>All wheat bread and rolls</td>
<td>Rye</td>
</tr>
<tr>
<td>White, wholemeal, multigrain, sourdough breads</td>
<td>Spelt</td>
</tr>
<tr>
<td>Cornbread</td>
<td>Buckwheat</td>
</tr>
<tr>
<td>Pitta bread, ciabatta, focaccia, panini, naan bread, chapatti, croissants, muffins, brioches, pastries, crumpets, English muffins, garlic bread, pizza</td>
<td>Millet</td>
</tr>
<tr>
<td><strong>Flour and raising agents</strong></td>
<td><strong>Noodles</strong></td>
</tr>
<tr>
<td>Wheat flour (white, wholemeal, plain, self-raising)</td>
<td>Rice noodles</td>
</tr>
<tr>
<td>Baking powder, bicarbonate of soda, cream of tartar, yeast</td>
<td>Buckwheat (soba) noodles</td>
</tr>
<tr>
<td><strong>Breakfast cereals</strong></td>
<td><strong>Savoury biscuits and snacks</strong></td>
</tr>
<tr>
<td>Wheat or bran based cereals (Weetabix, Shredded Wheat, bran flakes, All-bran, Cheerios, Fruit and Fibre)</td>
<td>Rice cakes and Snack A Jacks</td>
</tr>
<tr>
<td>Cornflakes, cornmeal porridge</td>
<td>Oatcakes</td>
</tr>
</tbody>
</table>

**Pasta**
- Fresh and dried wheat pasta (white, wholemeal), gnocchi
- Egg, Hokkein, Udon, Ramen, pot noodles, Supernoodles

**Flour**
- Rice flour
- Rye flour
- Spelt flour
- Buckwheat flour
- Millet flour

(minor amounts do not need to be avoided)

**Breakfast cereals**
- Rice Krispies and rice cereals
- Porridge oats and oat cereals
- Cereals and muesli containing unsuitable fruit
- Bircher muesli

**Biscuits or cakes made with oats, rice flour, spelt, buckwheat or millet**
- Cakes or biscuits containing unsuitable fruit

**Pastry**
- Pies, pastries containing unsuitable fruit

**Sweet biscuits and cakes**
- Flapjack

**Rice based meals**
- Sushi
- Fried rice
- Risotto
- Paella
### 8.14 Food diary guidance

#### Food and Drink Diary example

<table>
<thead>
<tr>
<th>Time</th>
<th>Food/drink description and preparation</th>
<th>Brand name</th>
<th>Portion size or quantity eaten</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Day of the week</strong></td>
<td><strong>Date</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Thurs</td>
<td>31 March</td>
<td></td>
</tr>
<tr>
<td>6.30 am</td>
<td>Filter coffee, decaffeinated Milk (fresh, semi-skimmed), Sugar (white)</td>
<td>Douwe Egberts</td>
<td>Mug A little 1 level tsp</td>
</tr>
<tr>
<td>7.30 am</td>
<td>Filter coffee with milk and sugar Cornflakes Milk (fresh, semi-skimmed) Toast, granary medium sliced Light spread Marmalade</td>
<td>As above Tesco's own Hovis Flora Hartleys</td>
<td>As above Picture 1b Drowned 1 slice Med spread 1 heaped tsp</td>
</tr>
<tr>
<td>9 am to 12 noon</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.15 am</td>
<td>Instant coffee, not decaffeinated Milk (fresh, whole) Sugar brown Digestive biscuit – chocolate coated on one side</td>
<td>Kenco</td>
<td>Mug A little 1 level tsp</td>
</tr>
<tr>
<td>11 am</td>
<td>McVitie’s</td>
<td></td>
<td>2</td>
</tr>
</tbody>
</table>

#### Pictures for food portion guidance

Use the pictures to help you indicate the size of the portion you have eaten. Write on the food record the picture number and size A, B or C nearest to your own helping.

Remember that the pictures are much smaller than life size. The actual size of the dinner plate is 10 inches (25cm), the side plate, 7 inches (18cm), and the bowl, 6.3 inches (16cm).

The tables in your food diary guidance booklet also give examples of foods that you might eat and how much information is required about them.

1. **Breakfast cereals**
   - Picture A
   - Picture B
   - Picture C

2. **Rice**
   - Picture A
   - Picture B
   - Picture C

3. **Spaghetti**
   - Picture A
   - Picture B
   - Picture C
Life size glass

![Life size glass image]

Life size spoons

- Teaspoon (5 ml)
- Dessert spoon (10 ml)
- Tablespoon (15 ml)

Participant Diary Follow up, Version 1, 27/02/2015
<table>
<thead>
<tr>
<th>Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1</td>
<td>Separate hard lumps, like nuts (hard to pass)</td>
</tr>
<tr>
<td>Type 2</td>
<td>Sausage-shaped but lumpy</td>
</tr>
<tr>
<td>Type 3</td>
<td>Like a sausage but with cracks on its surface</td>
</tr>
<tr>
<td>Type 4</td>
<td>Like a sausage or snake, smooth and soft</td>
</tr>
<tr>
<td>Type 5</td>
<td>Soft blobs with clear-cut edges (passed easily)</td>
</tr>
<tr>
<td>Type 6</td>
<td>Fluffy pieces with ragged edges, a mushy stool</td>
</tr>
<tr>
<td>Type 7</td>
<td>Watery, no solid pieces. Entirely Liquid</td>
</tr>
</tbody>
</table>
8.15 Example food diary day

<table>
<thead>
<tr>
<th>Time</th>
<th>Food/drink description and preparation</th>
<th>Brand name</th>
<th>Portion size or quantity eaten</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 am to 9 am</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 am to 12 noon</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>Food/drink description and preparation</td>
<td>Brand name</td>
<td>Portion size or quantity eaten</td>
</tr>
<tr>
<td>---------------</td>
<td>---------------------------------------</td>
<td>------------</td>
<td>--------------------------------</td>
</tr>
<tr>
<td>12 noon to 2pm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2pm to 5pm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>Food/drink description and preparation</td>
<td>Brand name</td>
<td>Portion size or quantity eaten</td>
</tr>
<tr>
<td>--------------</td>
<td>----------------------------------------</td>
<td>------------</td>
<td>--------------------------------</td>
</tr>
<tr>
<td>5pm to 8pm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8pm to 10pm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10pm to 6am</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Did you take any **vitamins, minerals or other food supplements** today?  
If yes, **please describe the supplements you took below**

<table>
<thead>
<tr>
<th>Brand</th>
<th>Name (in full) including strength</th>
<th>Number of pills, capsules, teaspoons</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Please record details of any recipes or ingredients of made up dishes or take-away dishes (if not already described).

<table>
<thead>
<tr>
<th>NAME OF DISH:</th>
<th>SERVES:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredients</td>
<td>Amount</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Brief description of cooking method
8.16 Example page from stool and symptom diary

Day of week: ........................................ Date: ........................................

1. Every time you open your bowels today please complete the relevant box(es) below. Please refer to the Bristol Stool Chart on the back page.

If you did not have a bowel action today please tick here: ☐

**BOWEL MOVEMENT 1**

Please indicate its consistency using the Bristol Stool Chart at the end of this booklet. *Please enter a value from 1 to 7.*

**BOWEL MOVEMENT 2**

Please indicate its consistency using the Bristol Stool Chart at the end of this booklet. *Please enter a value from 1 to 7.*

**BOWEL MOVEMENT 3**

Please indicate its consistency using the Bristol Stool Chart at the end of this booklet. *Please enter a value from 1 to 7.*

**BOWEL MOVEMENT 4**

Please indicate its consistency using the Bristol Stool Chart at the end of this booklet. *Please enter a value from 1 to 7.*

**BOWEL MOVEMENT 5**

Please indicate its consistency using the Bristol Stool Chart at the end of this booklet. *Please enter a value from 1 to 7.*

**BOWEL MOVEMENT 6**

Please indicate its consistency using the Bristol Stool Chart at the end of this booklet. *Please enter a value from 1 to 7.*
2. At the end of each day, please rate your symptoms by placing a tick in the box that best describes them. If you do not have this symptom, tick ‘absent’

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Absent</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal discomfort or pain (any kind of discomfort/ pain in your abdomen)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heartburn (burning / discomfort behind your breastbone)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid reflux / acid regurgitation (taste of sour fluid in your mouth)</td>
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<td>Nausea (feeling sick, but without vomiting)</td>
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<tr>
<td>Abdominal gurgling / rumbling (vibrations or noise in your stomach or belly)</td>
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<tr>
<td>Abdominal bloating / distension (swelling in your stomach or belly)</td>
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<tr>
<td>Belching / burping (bringing up gas through your mouth)</td>
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<td>Flatulence / passing wind (release of gas from your bottom)</td>
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<td>Constipation</td>
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<tr>
<td>Diarrhoea</td>
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<tr>
<td>Loose stools (mushy or watery stools)</td>
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<td>Hard stools (lumpy or dry stools)</td>
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<td>Urgency (urgent need to open your bowels)</td>
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<td>Incomplete evacuation (feeling of inability to pass all stool)</td>
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<td>Tiredness</td>
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<tr>
<td>Overall symptoms</td>
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Over the past seven days, do you feel that you have had adequate relief of your gut symptoms?

(i.e. think about your gut symptoms over the previous 7 days, do you feel your symptoms have been adequately controlled?)
8.17 Case-control study participant information sheet

Case-control study participant information sheet
IRAS ID: 203156
REC Reference Number: 16/LO/0976

Chief Investigator: Dr Majella O'Keeffe
Principal Investigator: Prof Kevin Whelan/Selina Cox

PARTICIPANT INFORMATION SHEET

(YOU WILL BE GIVEN A COPY OF THIS INFORMATION SHEET)

Dietary Intake in patients with Inflammatory Bowel Disease

We would like to invite you to participate in this research project. You should only participate if you want to; choosing not to take part will not disadvantage you in any way. Before you decide whether you want to take part, it is important for you to understand why the research is being done and what your participation will involve. Please take time to read the following information carefully and discuss it with others if you wish.

We are providing you with the following information before you agree to take part, which explains the purpose of this research and what it involves. Please ask any questions you have about the information given and we will do our best to explain and provide any further details you may need.

**What is the purpose of the study?**

This research study aims to help patients, doctors and dietitians at Guy's and St Thomas' NHS Foundation Trust and Barts Health NHS Trust to learn about the dietary habits of patients with inflammatory bowel disease compared to those without.

One of the aims of this study is to investigate the intake of a certain type of dietary carbohydrate in patients with inflammatory bowel disease, as previous studies have shown that patients with active inflammatory bowel disease may eat less of them. This may be important as these carbohydrates may affect gut symptoms and may also influence gut bacteria which are important in inflammatory bowel disease.

This study is being conducted as part of a PhD project in collaboration with Guy's and St Thomas' NHS Foundation Trust and Bart's Health NHS Trust and King's College London. We believe the results of this study will be important for the future treatment and dietary management of patients with inflammatory bowel disease.

**Who are we recruiting?**

We are looking for people with active or inactive inflammatory bowel disease (either Crohn’s disease or ulcerative colitis) who are attending an outpatient clinic at either Guy's Hospital, St Thomas’ Hospital or The Royal London Hospital as part of their care. People who have had medication dose changes, those who have had recent or extensive gut surgery or who have other psychiatric or chronic diseases will not be eligible. Pregnant and breastfeeding women will also be excluded.
We are also looking for people without inflammatory bowel disease, other gut conditions or serious conditions to take part in this study. This will allow us to compare the dietary intake of patients with inflammatory bowel disease to those without.

**Do I have to take part?**

No. Your participation in this study is voluntary and it is entirely up to you whether you take part. Even if you do decide to take part, you can change your mind at any time and withdraw from the study without giving a reason. If you decide you no longer wish to take part, please inform Selina Cox (contact details below). Any decision not to be involved in the study at any time will not affect the standard of care you receive now or in the future.

**What will happen to me if I do take part?**

This study involves completing a 7-day food diary which may be provided by the researcher on the day of your normal clinic appointment, if you wish to take part. After you have completed the diary, you will be asked to return it to the researcher at the hospital clinic or King’s College London (Waterloo), or you will be provided with a stamped envelope to return the diary via post if this is more convenient.

**Screening**

If you express interest in taking part in the study, you will be screened by a researcher to check whether you are eligible to take part and to ensure you meet all the inclusion criteria and none of the exclusion criteria. We will fully explain all the study procedures to you and you will be given at least 24 hours to consider the requirements of the study prior to signing the consent form, although this will need to be signed prior to starting the study. If you would prefer to sign the consent form and collect your food diary on the day of your clinic appointment (e.g. if it is more convenient) this will be possible.

**Questionnaire and food diary**

Once the consent form is signed you will be asked to complete a questionnaire regarding food-related quality of life and a questionnaire about your dietary habits. The food diary will then be explained in detail, and you will be asked to complete for the next 7-days. In this diary, you will be asked to record everything you eat and drink with estimated portion sizes. The diary will begin with instructions of how to complete it along with some portion size guides.

**Follow-up visit**

You may meet with the lead researcher at the hospital or King’s College London following completion of the 7-day food diary, to collect the diary and check that it is complete. This visit should take no longer than 15 minutes. Alternatively, you may use the stamped envelope provided to post the completed diary to the researcher, who may contact you via telephone to clarify any incomplete information in the diary.

**Expenses**

Reimbursements for expenses will not be available for this study.

**What are the possible disadvantages and risks of taking part?**

Completing the food diary may be burdensome. However, instructions and portion size guides are provided to make this process easier.

**What are the possible benefits of taking part?**
There are no immediate benefits to participants for taking part in this study. However, the results of this study may help to answer scientific questions about dietary intake in patients with inactive inflammatory bowel disease. This may therefore help you and other people in the future, as it may aid in the development of dietary interventions in inflammatory bowel disease.

**What if there is a problem?**

If you have a concern about any aspect of this study you should ask to speak to the researchers who will do their best to answer your questions (Selina Cox 0207 848 4552, selina.cox@kcl.ac.uk). Your inflammatory bowel disease will continue to be monitored by your gastroenterologist.

If you remain unhappy and wish to complain formally, you can do this through the Guy’s and St Thomas’ Patients Advice and Liaison Service (PALS) on 0207 188 8801, pals@gstt.nhs.uk. The PALS team are based in the main entrance on the ground floor at St Thomas’ Hospital and on the ground floor at Guy’s Hospital in the Tower Wing. At the Royal London Hospital, the PALS service is located on the 2nd floor Central Tower, near core lift 5 in the main building and can be contacted on 020 3594 2040, pals@bartshealth.nhs.uk.

In the event that something does go wrong, and you are harmed during the research then you may have grounds for legal action for compensation against King’s College London but you may have to pay your legal costs. King’s College London maintains adequate insurance to cover any liabilities arising from the study.

**Will my taking part be kept confidential?**

Your participation in the study would be completely confidential. From the beginning of your involvement, you will be given an identification number and only your number, NOT your name, will label your completed questionnaires, diaries and other paperwork. Your data will be kept completely anonymously and confidentially under the UK Data Protection Act 1998 and your name will not appear anywhere in any publication or description of our findings. Personal data and unidentifiable research data will be kept for 10 years on a research database.

**What will happen if I don’t want to carry on with the study?**

Taking part in this study is entirely voluntary and your decision will in no way affect your current or future care within this Trust. You will not lose any of your legal or ethical rights. You may withdraw from the study at any time without affecting your routine clinical care and you are not obliged to give reasons. If you withdraw from the study, we will need to use the data collected up to your withdrawal.

**What will happen to the results of the research study?**

It is intended that the results of this study may be published in scientific or medical journals. You will not be identified in any report or publication. When the data from the study has been analysed you will receive a summary report of the results.

**Who has reviewed the study?**

This study has been checked by an independent group of people, called a Research Ethics Committee to protect your safety, rights, well-being and dignity. This study has been reviewed and given favourable opinion by the NRES Committee: London City & East.

**Who is organising and funding the research?**
The study is organised and sponsored by King's College London (Diabetes and Nutritional Sciences Division, King's College London). The study is funded by internal funding. The Chief Investigator is Dr Majella O’Keeffe and the Principal Investigators are Prof Kevin Whelan and Selina Cox (King's College London).

If I’d like to participate, what should I do?

If you are interested in taking part or have any further questions or concerns at any time, then please call the contact number or write to the email address below. If you decide to take part, you will be given this information sheet to keep and will be asked to sign a consent form. Remember, if you do decide to take part you are still free to withdraw at any time and without giving a reason. You may also withdraw any data or information you have already provided up until it is transcribed for use in the final report.
### 8.18 Case-control study consent form

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<tr>
<td><strong>Participant ID:</strong></td>
<td><strong>Please initial the boxes</strong></td>
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<tr>
<td>1.</td>
<td>I confirm that I have read the information sheet dated............... (version..........) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily</td>
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</table>
| 2. | I consent to the following:  
   - Complete questionnaires as described in the information sheet  
   - Complete a 7-day food diary as described in the information sheet  
   - Return the diary to the researcher in person or via post |
| 3. | I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. |
| 4. | I consent to the processing of my personal information for the purposes explained to me. I understand that such information will be handled in accordance with the terms of the Data Protection Act 1998. |
| 5. | I understand that relevant sections of any of my medical notes and data collected during the study may be looked at by responsible individuals from regulatory authorities or from Guy’s and St Thomas’ NHS Foundation Trust or Barts Health NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. |
| 6. | I agree that any anonymised data collected in this study can be used in future research that has been approved by a recognised Research Ethics Committee. I am aware this research may be in collaboration with a commercial company, but that my identity will be kept anonymous. |
|   | Yes ☐ |
|   | No ☐ |
| 7. | I agree to take part in the above study. |
8.19 Stool collection instructions

Collecting your stool

During the study we will ask you to collect your stool at the baseline and follow-up visits. It is best that we collect your stool at the study centre if possible as we need to start processing it within 1-2 hours of being passed.

We gather important information from these stool collections, including information about the bacteria in your gut and pH.

Stool collection kit

1. Disposable paper plate
2. Clear plastic bag
3. Disposable gloves
4. Disposable plastic container
5. White bag

How to collect your stool

These instructions will also be given to you at your visit.

1. If you need to urinate, please do so before you collect your stool. YOU MUST ENSURE THAT YOU DO NOT URINATE AT THE SAME TIME AS COLLECTING YOUR STOOL SAMPLE - this would contaminate the sample.

2. Put on the disposable gloves.

3. Open the clear plastic bag
4. Place the bag inside the plastic pot and allow the handles to hang over the outside of the pot. Push the bag down inside the pot so that only the handles hang over the side.

5. Place the paper plate in the toilet bowl, and try to wedge it in slightly to keep it in place – this will stop the pot going in the water.

6. Sit the pot containing the plastic bag on top of the paper plate.
7. Pass your stool into the bags inside the pot. REMEMBER - YOU MUST ENSURE THAT YOU DO NOT URINATE AT THE SAME TIME as this would contaminate the sample. You may find it helpful to steady the pot with your gloved hand when initially passing the stool.

8. Remove the pot from the toilet bowl. Fold the bag loosely inside the pot on top of the sample. Put the lid on the pot and ensure that it is fully sealed.

9. Place the sample pot inside the white bag provided (and inside further bags if you wish) to transport to the study centre (if you are not collecting the sample at your visit).
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 improves abdominal symptoms in patients with inflammatory bowel disease—a pilot

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